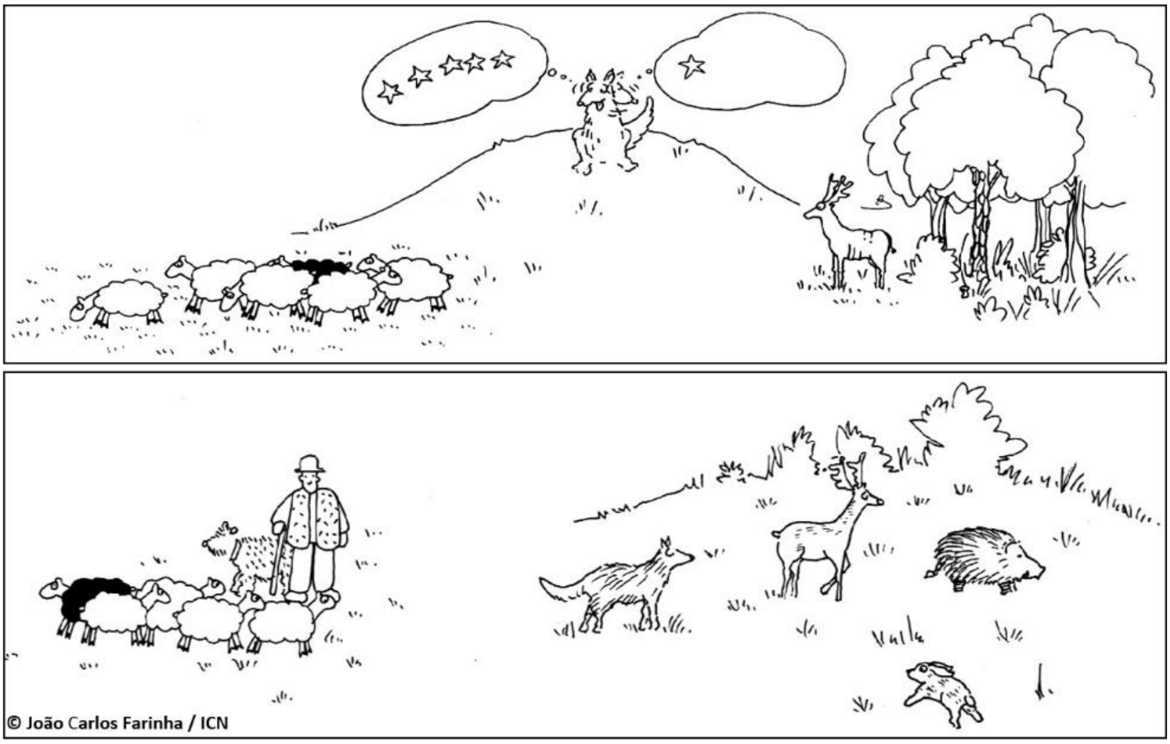

Wolf predation on livestock in relation to husbandry practices and wild prey availability: Regional and temporal patterns

Vera Joana Galego Casimiro

Mestrado em Biodiversidade, Genética e Evolução

Faculdade de Ciências, Universidade do Porto

Departamento de Biologia

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Tese orientada por:

Francisco Álvares

CIBIO/InBIO

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Todas as correcções determinadas
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MSc

“The wolf exerts a powerful influence on the human imagination.
It takes your stare and turns it back on you”
- Barry López, Of Wolves and Men

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ABSTRACT

Wolves, as key-stone species, affect biological communities, limiting them through predation and interspecific competition, influencing the nature and strength of ecosystem functioning. A specialized diet in ungulates is a common characteristic to wolves that often draws them into recurrent competition with humans, regarding the use of food resources, such as game or livestock. Throughout the world, conflicts of humans towards wolves due to livestock depredation undermine their mutual well-being, threatening, in turn, the conservation of many wildlife species involved. In this context, wolves have become targets of studies and management plans around the world.

Wolves are an extremely adaptable species showing a considerable breadth on diet, modifying their feeding habitats according with what is available, from large wild prey to domestic animals or even garbage. However, as a response to strongly anthropogenic environments, in many regions livestock turned to be a substantial part of wolf's diet, which is consumed either by scavenging or predation as main feeding behaviour. Considering the difficulties wildlife managers must face when dealing those conflicts, one of the first steps is to characterize wolf biology and, in particular, trophic ecology as a function of various interrelated variables, such as habitat conditions, prey availability and feeding strategies. However, despite several existing studies regarding the feeding habits of this carnivore worldwide, few have addressed multi-prey systems dominated by domestic animals such as the one occurring in Portugal and to which there is a lack of updated information on wolf diet at a national level.

The goal of this study was to address wolf trophic ecology, such as diet, prey selection and feeding behaviour using Portuguese wolves as a model, evaluating the influence of livestock husbandry practices and wild prey availability in wolf feeding ecology, considering regional, temporal and even individual patterns of variation (e.g. intra pack variation and individual variation along time). For that purpose, information was obtained from three different study areas within wolf range in Portugal (Montesinho, South Douro and Peneda-Gerês). A total of 307 scats genetically confirmed as being from wolves, distributed through the three study areas, were analysed using standardized procedures to identify hairs and other macroscopic remains of prey items, followed by data analysis considering prey availability, official statistics of wolf attacks to livestock and information from previous studies on wolf diet in each study area. Global results of wolf diet expressed in Frequency of Occurrence and consumed Biomass revealed different feeding habits across the three wolf population nuclei in

Portugal, in particular the proportion in consumption of domestic and wild prey, reflecting the diversity and availability of prey species in each study area. In Montesinho study area, three species of wild ungulates comprise currently 90% of consumed Biomass with a steady and sharp increase of wild prey consumption along the last few decades and a strong positive selection towards red deer. As for Peneda-Gerês, two species of livestock in free-ranging husbandry with poor vigilance, cattle and particularly horses (also with a strong positive selection), comprised up to 90% of consumed Biomass, a value that has been relatively constant over the last decades and result in the highest values of damage compensation attributed to wolves at a national level. In Sul do Douro study area, wolves are currently highly dependent on the consumption of Lagomorphs, a prey item that represents almost 50% of consumed Biomass, and with a clear increase during the last years followed with a major decrease on consumption of domestic ungulates. This result suggests a feeding behaviour based on scavenging of domestic rabbit carcasses from intensive production farms. Concerning patterns of individual variation on wolf diet, despite a small sample size of scats with molecular individual identification, this preliminary approach suggested different prey preference and foraging behaviours among individuals belonging to the same pack as well as in the same individual during a long time period. This study provided valuable insights on wolf trophic ecology in a multi-prey system dominated by domestic species, revealing particularities of the wolf diet for each study area according to livestock husbandry practices and wild prey availability. Furthermore, preliminary results of individual variation on wolf diet revealed an enormous potential of this topic as a research avenue, considering it is an unexplored field of carnivore ecology worldwide that can provide important implications regarding wolf management. Finally, this study provides support for several management actions to be conducted in Portugal (e.g. reinforcement of wild ungulates populations, promote preventive measures to reduce livestock losses, regulating wolf access to carrion) in order to assure the long-term survival of wolves in human-dominated landscapes.

Key words:

Wolf trophic ecology, Multi-prey system, Scavenging, Scat analysis, Diet shift, Prey selection, Feeding behaviour

RESUMO

Sendo espécies chave, os lobos afectam as comunidades biológicas, limitando-as através da predação e competição intra-específica, influenciando a natureza e a força do funcionamento do ecossistema. Uma dieta especializada em ungulados domésticos é uma característica comum entre carnívoros que frequentemente os leva para competições recorrentes com humanos, que apresentam necessidades semelhantes no que diz respeito ao uso de recursos biológicos, como animais de caça e de gado. Em todo o mundo, conflitos de humanos direccionados a grandes carnívoros devido à predação em gado, põe em causa o seu mútuo bem-estar, ameaçando, por sua vez, a conservação de muitas espécies selvagens envolvidas no sistema. Neste contexto, os lobos tornaram-se alvos de estudo e planos de conservação pelo mundo e ao longo de vários anos.

Como uma espécie extremamente adaptada e verdadeiramente generalista, os lobos demonstram uma considerável amplitude de dieta, modificando-a de acordo com o que encontram disponível, desde grandes presas selvagens a presas domésticas, ou até mesmo lixo. Contudo, como resposta e adaptação a fortes condições antropogénicas, em muitos locais, as presas domésticas tornaram-se a parte substancial da ecologia trófica do lobo, tanto a nível de necrofilia como de predação, trazendo por sua vez os principais problemas no que concerne os conflitos entre humanos e lobos. Considerando as dificuldades que os conservacionistas de vida selvagem enfrentam quando lidam com esses conflitos, um dos primeiros passos é caracterizar a biologia do lobo e, em particular, a sua ecologia alimentar como um funcionamento de várias variáveis correlacionadas, como as condições de habitat, a disponibilidade de presas e as estratégias alimentares. Contudo, embora tenham sido realizados vários estudos no que diz respeito aos hábitos alimentares deste carnívoro, poucos são os que abordam os sistemas de multi-presas dominados por animais domésticos, como o que ocorre em Portugal, e no qual há uma falta de informação actualizada da dieta de lobo a nível nacional.

O objectivo deste estudo foi explorar a ecologia trófica do lobo, como a dieta, selecção de presas e o comportamento alimentar, usando os lobos Portugueses como modelo, avaliando a influencia dos sistemas de pastoreio e a disponibilidade de presas silvestres, considerando padrões regionais, temporais e individuais (e.g. variação intra-alcateia e variação individual ao longo do tempo). Para esse efeito, foi obtida informação de três diferentes áreas de estudo dentro da distribuição do lobo em Portugal (Montesinho, Sul do Douro e Peneda-Gerês). Foi analisado um total de 307

dejectos geneticamente confirmados como sendo de lobo, distribuídos pelas três áreas de estudo, usando procedimentos padronizados para identificas pêlo e outros materiais macroscópicos das classes-presa, seguido de uma análise de dados da informação obtida, considerando disponibilidade de presas, estatísticas oficiais de ataques de lobo sobre gado e informação de estudos prévios em dieta de lobo em cada área de estudo.

Os resultados globais expressos em Frequência de Ocorrência e Biomassa consumida revelaram diferentes hábitos alimentares nos três núcleos de populações lupinas em Portugal, em particular a proporção em consumo de presas domésticas e silvestres, reflectindo a diversidade e disponibilidade de espécies de presa em cada área. Na área de estudo de Montesinho, três espécies de ungulados correspondem actualmente a 90% da Biomassa consumida, com um elevado e acentuado aumento no consumo de presas selvagens ao longo das últimas décadas, e uma forte selecção positiva sobre veado. Quanto à área de estudo de Peneda-Gerês, duas espécies de animais domésticos em sistema livre de pastoreio com pouca vigilância, vacas, e em particular cavalos (também com uma forte selecção positiva), constituem cerca de 90% da Biomassa consumida, um valor que tem sido relativamente constante ao longo dos últimos anos e um resultado dos elevados valores de compensações atribuídas devido a danos causados pelo lobo a nível nacional. Na área de estudo do Sul do Douro, a população lupina está actualmente dependente do consumo de Lagomorfos, uma presa que representa aproximadamente 50% da Biomassa consumida, e com um evidente aumento durante os últimos anos, seguido por um grande decréscimo no consumo de ungulados domésticos. Este resultado sugere um comportamento alimentar baseado na necrofagia de carcaças de coelhos domésticos provenientes de sistemas intensivos de produção. No que diz respeito aos padrões de variação individual na dieta do lobo, embora seja uma pequena amostra de dejectos com identificação molecular individual, este estudo preliminar sugere diferentes preferências a nível de consumo de presas e um comportamento de “foraging” entre indivíduos pertencentes à mesma alcateia, assim como no mesmo indivíduo durante um longo período de tempo. Este estudo providenciou informações muito importantes relativamente à ecologia trófica do lobo para cada área de estudo, de acordo com diferentes sistemas de práticas pastorícias e disponibilidade de presas domésticas. Além disso, os resultados preliminares da variação individual da dieta do lobo revelaram um enorme potencial neste tópico como área de investigação, considerando ser ainda um campo por explorar na ecologia dos carnívoros pelo mundo, e por isso, podendo providenciar importantes implicações no que diz respeito à gestão do lobo. Finalmente, este estudo proporciona suporte para diversos planos de conservação a

serem conduzidos em Portugal (e.g. reforço de ungulados selvagens, medidas preventivas de redução das perdas de gado, regulação do acesso do lobo a carcaças) de forma a assegurar uma sobrevência a longo prazo da população lupina nas áreas dominadas pelo Homem.

Palavras-Chave:

Ecologia trófica do lobo, Sistema de multi-presas, Necrofagia, Análise de dejectos, Mudança de dieta, Selecção de presas, Comportamento alimentar

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LIST OF ABBREVIATIONS

Biomass – Consumed Biomass

CIBIO – Research Centre of Biodiversity and Genetic Resources, University of Porto

F.O. – Frequency of Occurrence

GIS – Geographic Information Systems

GPS – Global Positioning Systems

H' – Shannon's diversity index

ICNF – Institute for Nature Conservation and Forest

N.B. – Niche breadth

NE – Northeast

N.O. – Niche overlap

NW – Northwest

QGIS – Quantum Geographic Information System

CHAPTER I
INTRODUCTION

LARGE CARNIVORES: ECOLOGICAL ROLE AND CONFLICTS WITH HUMANS

Large carnivores, as top predators, play an important and structuring role in regulating many ecosystems, exerting a profound influence on biological communities and in maintaining the balance (Terborgh *et al*., 1999; Linnell *et al*., 2000; Miller *et al.,* 2001; Ripple *et al.,* 2014). Their presence is considered a "stamp of quality" that certifies the integrity, sustainability and health of larger ecosystems worldwide (Maheshawari *et al.,* 2015). As keystone species, large carnivores affect biological communities, limiting them through predation and intraspecific competition, thus influencing the nature and strength of ecosystem functioning (Mills, 1991; Schmitz *et al*., 2000; Miller *et al*., 2001; Treves and Karanth, 2003; Williams *et al.*, 2004; Ripple *et al.,* 2014). As an example, when selecting from a wide-range of prey species, carnivores enforce ecological boundaries, affecting the dynamics of herbivores and reducing the amount of pressure they place on lower levels of the trophic chain, thus increasing the production and biodiversity of autotrophs. When the effects of these top consumers are reduced or removed, it constitutes a threat to biological diversity (Berger *et al.*, 2001; Estes *et al.*, 2011).

A specialized diet in ungulates is a common characteristic to many large carnivores, therefore, beside wild prey, carnivores may also kill domestic ungulates whenever opportunities arise. However, this often draws them into recurrent competition with humans, who have, more or less, similar needs regarding the use of biologic resources, such as game or livestock (Meriggi and Lovari, 1996; Treves and Karanth, 2003; Schwedner and Gruber, 2007; Odden *et al*., 2013). The confrontations of carnivores' intrinsic biology (e.g. low population densities, low reproductive rates, long periods of gestation, high food requirements and wide-ranging behaviour) with anthropogenic threats, makes them vulnerable and poorly able to respond and recover from external threats and possible extinction (Cardillo *et al.*, 2004; Ripple et al, 2014). Throughout the world, conflicts and superstitions of humans towards large carnivores undermine their mutual well-being, threatening, in turn, the conservation of many wildlife species involved (Madhusudan, 2003). Considering the current unfavourable situation of most populations of large carnivores, conservation plans are now part, and a priority, of the majority of studies targeting these species. Yet, it may be a challenging task, considering their large home ranges (Treves and Karanth, 2003; Maheshawari *et al.,* 2015).

By exploiting their charisma, top predators are frequently used by conservation biologists as flagship or umbrella species to acquire public and financial support for conservation actions, raise environmental awareness and planning protected areas (White *et al*., 1997; Carrol *et al*., 2001; Sergio *et al*., 2006). Preservation of top predators is a very delicate and complex task, and the increase of the implicit economic costs often constraint the acceptance of conservation actions (Sergio *et al.*, 2003). Ideally, an affected community would manage human-wildlife conflicts without permanently damaging biodiversity (Treves *et al*., 2006), but that is still a distant scenario worldwide. As an incentive for predation tolerance towards carnivore predation on game or domestic species, compensation programs have sought to minimize and offset monetary costs to humans, in order to reduce the economic impact of losses from carnivore predation. In many worldwide areas, people suffering losses from carnivores are able to claim compensations for damages to livestock, crops and other properties (Madhusudan, 2003; Hemson *et al*., 2009). However, there is an incentive-compatibility problem; unless compensation programs provide explicit incentive to encourage better livestock protection and more tolerance over carnivores, it can result in the neglect of preventive measures (Hemson *et al*., 2009). The optimal compensation mechanism is when the potentially affected parties are compensated for their precautionary efforts rather than for their livestock losses. Compensation programs require ecological knowledge on carnivore prey-interactions (such as predation impact and prey selection), as well as well-defined goals, and need to be more realistic and responsive if there are to help relieving the conflict (Dyar and Wagner, 2003; Madhusudan, 2003; Maheshawari *et al.,* 2015). At the very least, they should mitigate the incentive to kill carnivores for defensive or revenge reasons (Dyar and Wagner, 2003; Bulte and Rondeau, 2005).

The conflict between carnivores and humans due to livestock depredation is a worldwide problem very well exemplified by wolves (*Canis lupus*), one of the world's most widely distributed mammal and the most studied large carnivore (Ripple *et al*., 2014). Therefore, considering the variable ecology of this species across regions and the historical relation it holds with humans, in the last decades, wolves have become targets of studies and conservation plans around the world, even though it may be translated into rough and true challenges when it comes to achieve a perfect balance between humans and wildlife.

THE WOLF AS A CASE STUDY: RELEVANCE OF LIVESTOCK DEPREDATION

Wolves are an extremely adaptable species, capable of surviving anywhere where they are not killed by humans and where there is something to eat, from large wild prey to domestic animals or even garbage (Boitani, 2000). For the last thousands of years, wolves have lived alongside with humans, throughout the Northern hemisphere, occupying a special place in the relationship between their natural environment and Man. However, when humans started to live dependent of their crops and livestock, this predator became a threat. Similar social systems, hunting needs and techniques, territoriality and ecological flexibility made the wolf a companion but also a competitor and a burden (Boitani, 2000). Hence, for centuries, the wolf has been associated with a negative image, representing one of the greatest conflicts between humans and wildlife (Boitani *et al.*, 2010).

The wolf has been a target of several studies throughout the world and along several years (Boitani, 2000; Miller *et al*., 2001; Mech and Boitani, 2003; Ripple *et al.,* 2014). It is a generalist species when it comes to habitat requirements, adapted to all types of environment and living in the most diverse and extreme habitat conditions (Mech, 1995; Mech and Boitani, 2003).

When a pair of wolves establishes a territory and reproduces, it consists in a social unit denominated by pack, originating a numerous and structured group. This group lives according a hierarchy, composed by the alpha breeding pair, considered the pack leaders, their pups, and a variable number of nonbreeding males and females, frequently from previous offspring (Boitani, 2000; Peterson *et al.,* 2002; Cipponeri and Verrell, 2003). Among individuals there are strong social bonds that co-operate with each other, regulating together the internal stability and the dynamic of the pack, hunting strategically and defending their territory from intruder wolves (Boitani, 2000). Most wolves belong to packs and usually operate as a group. However, about 8% to 28% of them sometimes split off as smaller packs or individually (Mech, 1970). This may happen when they mature and disperse from their birth pack, or as a result of foraging behaviour. When prey are scarce, there are better probabilities for a wolf to find more food when on its own (Schmidt and Mech, 1997; Mech, 1999). Regarding feeding behaviour, it is known that after a kill, wolves consume the highly nutritious organs first, followed by the major muscle masses and eventually bones and skin (Stahler *et al.*, 2006). In this matter, being the breeding pair is translated into having certain privileges. This rank allows the dominate individuals to gain advantageous access to food and the choice of to whom to allot it (Mech, 1999). However, little is known about the feeding variation among packs or dispersant wolves along time.

Considering the relation between the size of the pack with the size of the prey (Thurber and Peterson, 1993; Schmidt and Mech, 1997; Hayes *et al*., 2000; Jedrzejewski *et al*., 2002), the only thing expected is for a “lone wolf” to hunt smaller prey, like domestic animals, in comparison with a pack, that working as a group, are able to kill larger prey, like deer.

Although wolves are primarily predators of ungulates, they are true generalists, showing a considerable breadth of diet, modifying it according with what is available (Peterson and Ciucci, 2003; Darimont *et al*., 2003). Feeding opportunistically on what is within reach, wolf diet varies from large wild prey, such as deer and wild boar *(Sus scrofa*), to small prey, such as lagomorphs and rodents, or even carcasses, human waste and fruit. In areas with lack of their natural prey, takes place the depredation over domestic animals. This is as old as domestication itself and is the most serious issue in wolf conservation (Boitani, 2000). Along with the conflict with human economies, livestock depredations are the main causes for wolf control or extermination (Meriggi and Lovari, 1996; Boitani, 2000; Hosseini-Zavarei *et al*., 2013; Llaneza and López-Bao, 2015). Moreover, the lack of an efficient guarding system to livestock aggravates the problem and enables recurrent attacks and significant losses. As a way to diminish the conflict, traditional defence strategies against wolf predation, combining different prevention measures, are still used in several countries (Boitani *et al*., 2010). For millennia, livestock guarding dogs have proved to be an effective mean in protecting rangelands and livestock from predators (Otstavel *et al*., 2009). Alongside with guarding dogs, shepherds, night-time enclosures and other husbandry practices are also good systems for predator control (Kaczensky, 1999). In parallel with good guarding systems to livestock, an abundant and diverse population of wild prey, represents an effective contribution for lowering predation on domestic prey (Meriggi and Lovari, 1996).

Wolf populations and pastoralism economies have always been in conflict. As a response and adaptation to strongly anthropogenic conditions, in many regions, livestock is a substantial part of wolf’s trophic ecology, both by scavenging or predation (Salvador and Abad, 1987; Ciucci and Boitani, 1998; Vos, 2000; Gazzola *et al*., 2008; Iliopoulos *et al.,* 2009; Boitani *et al*., 2010). Management and conservation implications of this issue are particularly relevant in places where wolf depredation affects livestock species with high socio-economic value (Pimenta *et al*., 2017). In European countries, where wolf’s natural habitat and wild prey populations of the wolf have been continuously altered by a long history of human development, the survival of this top predator is still very dependent on livestock (Ciucci and Boitani, 1998). Hence, compensation is provided with the aim of alleviate social tension and support farmers who have losses, although it is far from being the solution to the problem (Boitani, 2000; Treves *et al.,* 2003; Boitani *et al.*, 2010). Since human societies started to preserve their wolves, populations have increased and they are successfully returning to the lands they were exterminated long ago (Chapron *et al*., 2014). The re-establishment of suitable ecological conditions has also contributed to wolf recovery, and is starting to have a big impact in some wolf areas. As an opportunistic predator, the wolf consumes the most abundant prey species. With the growth of wild ungulates populations, they are becoming an easier prey and wolves are positively responding to that by shifting their feeding habits (Gazzola *et al*., 2007; Barja, 2009).

Considering the difficulties wildlife managers must face when dealing with human-wolf conflicts, one of the first steps is to characterize wolf biology and, in particular, feeding ecology as a function of various interrelated variables, such as habitat conditions, prey availability and feeding strategies (Boitani, 2000). More recently, molecular tools became available and important, extensively used, facilitating conservation actions and allowing the knowledge of crucial biological aspects of the wolf, such as feeding habits based on scat analysis. The application of non-invasive genetics on dietary studies allows species identification to reduce the bias of scat misidentification among sympatric species of the wolf (Monterroso *et al*., 2013), namely the dog (*Canis familiaris*) or the fox (*Vulpes vulpes*). Furthermore, as already done in studies of other carnivores (Mondol *et al.*, 2009), it allows individual identification of wolf scats, enabling to access individual variations of feeding habits.

The conservation of this top predator requires an enormous challenge, particularly important in regions where wolves live in habitats that are highly humanized, such as the Iberian Peninsula and particularly Portugal.

THE WOLF IN PORTUGAL: POPULATION STATUS AND TROPHIC ECOLOGY

The Iberian Peninsula is home of a sub-species of grey wolf, the Iberian wolf (*Canis lupus signatus*). For centuries, this subspecies had optimal conditions for its survival, with human presence being scarce and wild prey populations existing in high densities. During this time, wolf range occupied almost the whole area of the Peninsula (Blanco *et al.,* 1992; Pimenta *et al.,* 2005;). However, in the early 20th century, due to a systematic and intense human direct persecution and major habitat changes, particularly in forest cover and wild prey availability, distribution and abundance of wolf populations started to decline, with this species becoming extinct from West to East and from South to North of the Iberian Peninsula. Today wolves only persist in

Northwest Iberia and in a small isolated area in Southern Spain (Blanco *et al.,* 1992; Grilo *et al.,* 2002; Álvares, 2004).

In Portugal, wolves still existed almost in all national territory, until the beginning of the 20th century, but since the decade of 1930 this species has progressively become extinct in many areas, namely in south, coastal and central regions of the country (Figure 1).

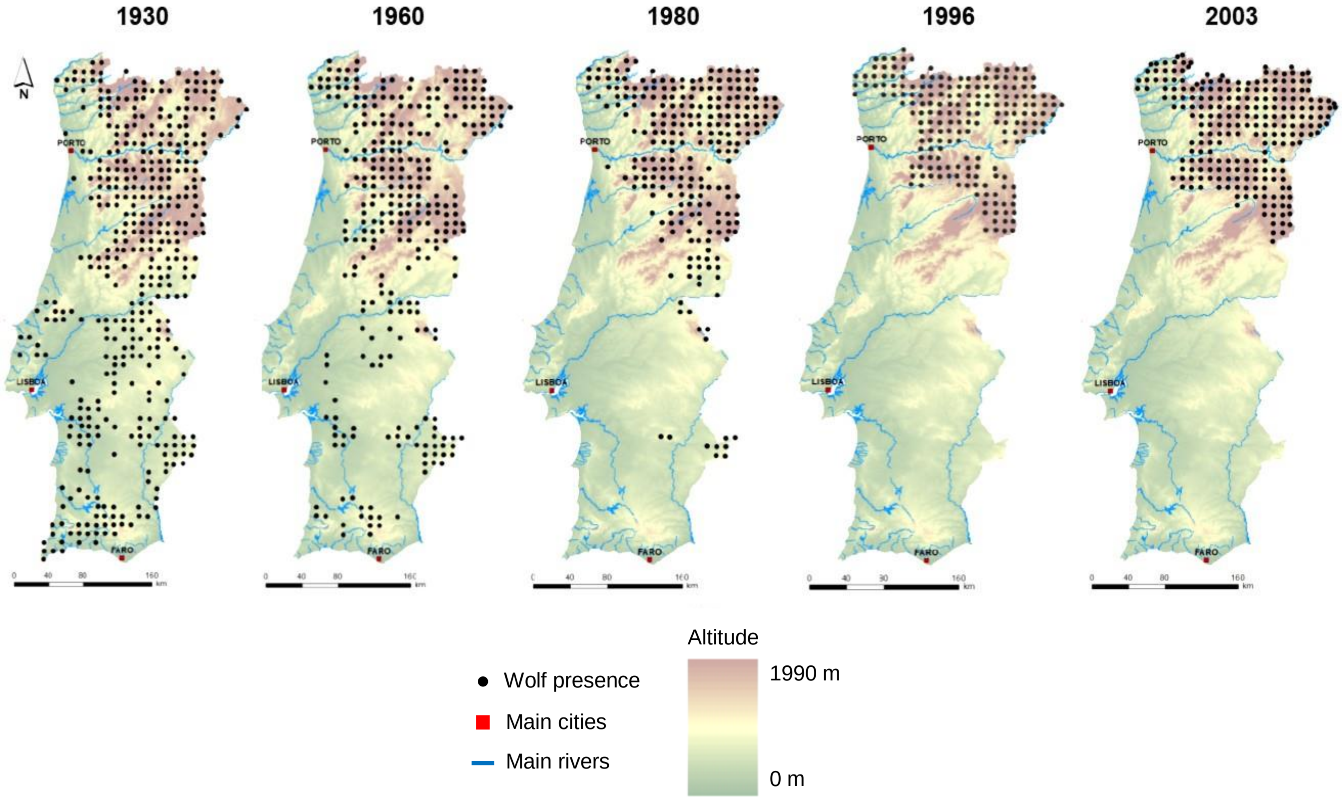


Figure 1 – Wolf distribution in Portugal between 1930 and 2003. Source: Álvares *et al.,* 2015.

As a consequence of the dramatic decrease in wolf numbers and distribution during most 20th century, this carnivore became fully protected by specific legislation since 1988 (Law 90/88, regulated by the decree-law 139/90). This legislation forbids wolf killing, habitat destruction and disturbance, especially during wolf breeding season, and includes a compensation programme for wolf damages to livestock, conducted by the Institute for Nature Conservation and Forests (ICNF), the governmental institution responsible for wolf conservation and management in Portugal (Grilo *et al.*, 2002; Bessa-Gomes and Petrucci-Fonseca, 2003; Álvares, 2004; Pimenta *et al.*, 2005). As a result of legal protection, since late 1990s, wolf range have stabilized, but this species is still listed as “Endangered” in the Portuguese Red Data Book, and in some areas, local extinctions may still occur (Pimenta *et al.*, 2005; Eggermann *et al.,* 2011). Today, wolves only persist in about 20% of their original range, occurring in the Northern mountain areas of the country, characterized by low human density, but with important agriculture and livestock husbandry practices (Petrucci-Fonseca, 1990; Bessa-Gomes and Petrucci-Fonseca, 2003; Pimenta *et al*., 2005; Álvares *et al*., 2015). Based in the last national wolf census conducted in 2002/2003, wolf population in Portugal was estimated in approximately 300 individuals, comprising 65 breeding packs (Pimenta *et al*., 2005). Currently, the main and more stable wolf population nuclei of Portugal are located in Peneda-Gerês National Park (Northwest), Montesinho Natural Park (Northeast) and Alvão Natural Park (central North Portugal) (Figure 2). These three population nuclei located North of the Douro river are of major importance and influence when it comes to maintain wolf packs in the more unstable surrounding areas (Álvares, 2004; Pimenta *et al.*, 2005). Besides, a small and isolated wolf population persists in the South region of the Douro river, comprised by less than 10 packs with low breeding rates, showing a high degree of fragmentation and facing a serious risk of extinction (Grilo *et al*., 2002; Grilo *et al*., 2004).

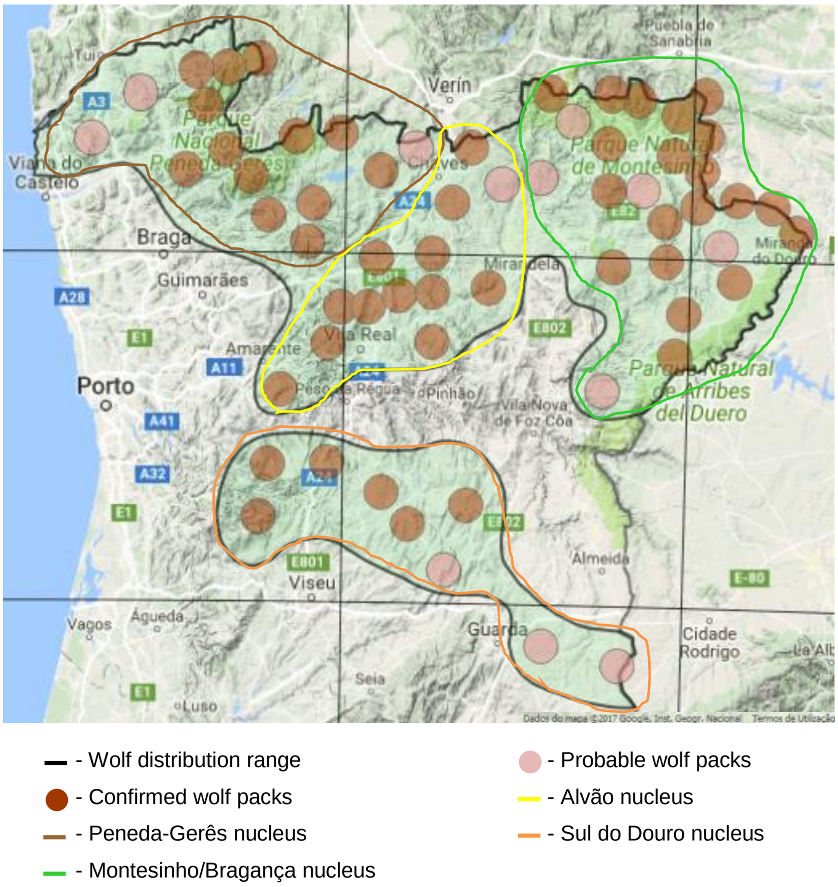


Figure 2 – Wolf distribution range in Portugal, representing the four main populations nuclei and the location of confirmed and probable packs, according to the last Wolf National Census conducted in 2002/2003 (Pimenta *et al.*, 2005).

Portugal has a highly anthropogenic landscape, where most of the former wolf habitat has increasingly become deforested, degraded and with absence or scarcity of wild prey (Santos *et al*., 2007; Eggerman *et al*., 2011). As a result, wolves rely almost completely on domestic animals, particularly extensive grazing livestock, similarly to other regions of Southern Europe (Zlatanova *et al.,* 2014). In fact, throughout almost all wolf range in Portugal, livestock comprises most of wolf diet (Petrucci-Fonseca, 1990; Carreira and Petrucci-Fonseca, 2000; Vos, 2000; Álvares *et al.,* 2015; Torres *et al.,* 2015a). However, despite wolves having a small range in Portugal, there are marked regional differences in the proportion of livestock consumption as well as the relevance of each livestock species in wolf diet, as revealed by dietary studies based on scat analysis mostly conducted some decades ago (Figure 3) (Álvares, 2004).

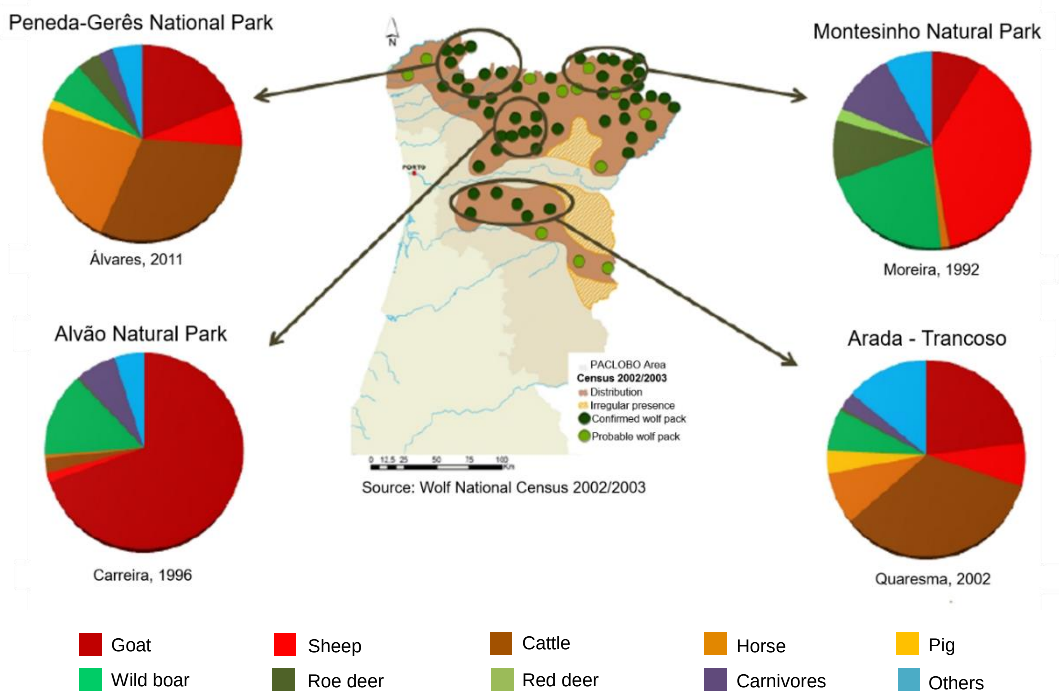


Figure 3 – Regional variation of wolf diet: Frequency of occurrence of food items in four areas from the main wolf population nuclei. Source: Álvares *et al*., 2015.

In Peneda-Gerês National Park (NW Portugal), livestock, specially Garrano horses (*Equus caballus*) and cattle (*Bos taurus*), generally roams free and unprotected in the mountains during all year and constitutes up to 90% of wolf diet (Petrucci-Fonseca, 1990; Álvares *et al.,* 2000; Vos, 2000; Pimenta *et al*., 2017). Consequently, this is the region with the highest values of wolf depredation on livestock in Portugal, which originates huge conflicts between humans and wolves, resulting in illegal persecution to wolves by shooting, poison or snare (Álvares *et al.,* 2000; Pimenta *et al*., 2005; Álvares, 2011). Notwithstanding, due to the low human density in Peneda-Gerês mountainous region and the considerable availability of food (e.g. livestock biomass), wolves are still capable of maintaining a stable and high population density (Álvares *et al.,* 2000). In an opposite ecological scenario, in Montesinho Natural Park (NE Portugal), a rich community of wild prey – red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and wild boar – occur in high densities and constitute wolves main prey, representing up to 70% of wolf diet (Petrucci-Fonseca, 1990; Moreira, 1992; Pimenta, 1998). This fact allows the maintenance of high population densities of wolves, considering that this is the nuclei that cause less impact on livestock, and consequently suffering less from human persecution (Pimenta *et al.,* 2005). As for Alvão mountains (central North Portugal) and South Douro river region, wolves show an intermediate ecological situation when compared with the previous two nuclei. In Alvão and South Douro, wolves present a similar ecological behaviour, feeding mainly on the two most common ungulates in the area, goats (*Capra hircus*) and wild boar (Carreira and Petrucci-Fonseca, 2000; Álvares, 2004; Torres *et al*., 2015a). Moreover, in the South Douro wolf population, some pack frequently feed on garbage dumps and carrion from carcasses that are dumped by the cattle industry, pig or rabbit farms and poultries (Álvares *et al*., 2015; Alexandre *et al*., 2000; Vos, 2000; Grilo *et al*., 2004). Although the dependence on livestock by Portuguese wolves is very high, the presence of domestic species in wolf diet does not necessarily reflect a predatory behaviour as feeding strategy, as shown by South Douro wolf population. Instead, livestock may be consumed by a scavenging behaviour resulted from the availability of carcasses of domestic animals raised both in extensive grazing or intensive farming. This topic has important management and sanitary implications, although it has been poorly addressed on studies focusing Iberian wolf trophic ecology (Llaneza and López-Bao, 2015).

As a result of a diet based on livestock, wolves in Portugal are responsible for high numbers of attacks to domestic animals and, consequently, for a significant amount of compensation values to livestock depredation paid by the national authorities (ICNF). Damage compensation started at national level in 1990, after wolf legal protection (Pimenta *et al*., 2005). Between the decade of 1990 and 2001, the number of livestock attacks attributed to wolves and the respective amount spent in damage compensation had an increasing rate (Figure 4), reflecting a higher awareness of livestock owners to the right of having their damages compensated rather than a trend in wolf numbers (Pimenta *et al*., 2005; Álvares *et al*., 2015). Since early 21th century, damage compensation values have stabilized and, currently, the annual amount of compensation paid for wolf damages to livestock at a national level is about 750.000€, corresponding to approximately 2.500 attacks per year attributed to wolves (Álvares *et al*., 2015) (Figure 4).

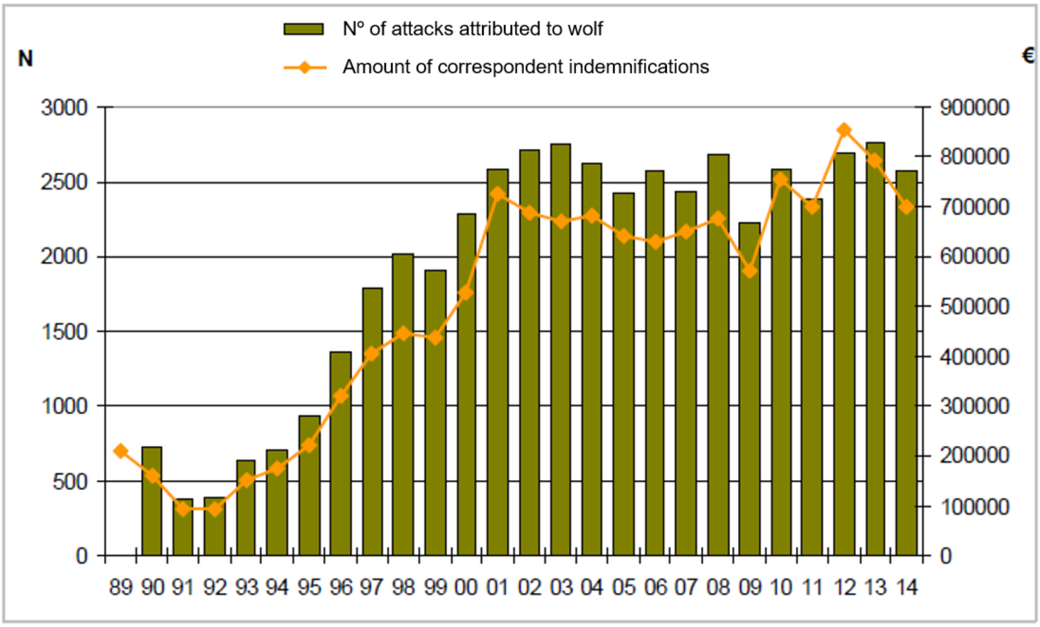


Figure 4 – Temporal evolution of the annual number of attacks to livestock attributed to wolf and respective amount compensation by National authorities (ICNF), between 1989 and 2014. Source: Álvares *et al.,* 2015.

There are regional differences on the magnitude and trends of the annual number of wolf attacks to livestock in Portugal, leading to heterogeneity in compensation values attributed due to livestock losses and reflecting the ecological characteristics in each wolf population nuclei as described above. The region of Peneda-Gerês has, by far, the highest values of wolf attacks to livestock declared at national level, and together with Alvão and South Douro nuclei, had an increasing trend during the last years (Figure 5). In opposite, Montesinho/Bragança has been recording fewer numbers of attacks to domestic animals attributed to wolves (Figure 5). These regional and temporal differences may be due to several reasons, such as changes in wolf numbers,

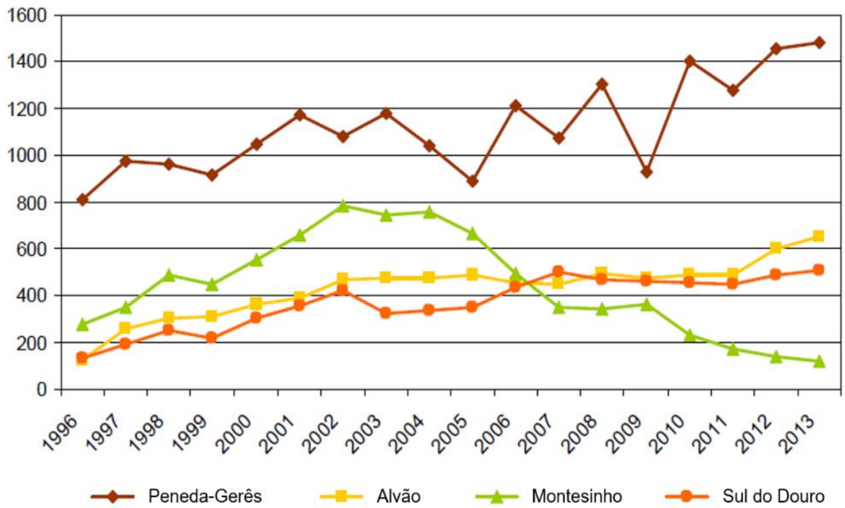


Figure 5 – Temporal evolution of the annual number of attacks to livestock attributed to wolf in the four wolf population nuclei in Portugal (Peneda-Gerês, Alvão, Montesinho and Sul do Douro), between 1996 and 2013. Source: Álvares *et al*., 2015.

in wild prey availability and/or in husbandry practices and prevention measures for livestock towards wolf predation.

Regarding the possible influence of wolf numbers and trends in recent variations on livestock damages, the lack of a recent wolf population census at national or regional level precludes the accurate evaluation of this effect, although wolf population in Portugal is considered to be stable in the last few years (Álvares *et al*., 2015). On contrary, there are several documented changes in wild prey availability and livestock husbandry practices that may be influencing these recent trends in the magnitude of wolf attacks to livestock. The availability of wild prey as a stable food resource for wolves is an important aspect affecting wolf predation on livestock, since several studies have shown that livestock depredations decrease in areas with higher diversity and densities of wild prey (Meriggi and Lovari, 1996; Peterson and Ciucci, 2003; Odden *et al*., 2013; Torres *et al*., 2015a). In Portugal, populations of wild boar, roe deer and red deer that were practically exterminated in the early 20th century, have been increasing in range and density in the last few decades (Salazar, 2009; Vingada *et al*., 2010; Álvares *et al*., 2015; Torres *et al*., 2015b). Within wolf range, natural populations of wild boar are currently widespread and at high densities while roe deer occurs mainly at north of Douro river apparently with low densities. Red deer is mostly restricted to a small range locates in NE Portugal, particularly in Montesinho Natural Park (Figure 6) (Vingada *et al*., 2010; Álvares *et al*., 2015). Moreover, recent reintroductions of some wild ungulates, such as roe deer at South Douro river region, as well as wild goat (*Capra pyrenaica*) and red deer at Peneda-Gerês National Park, are increasing the availability and diversity of wild prey within the range of several wolf packs (Moço *et al*., 2006; Álvares *et al*., 2015).

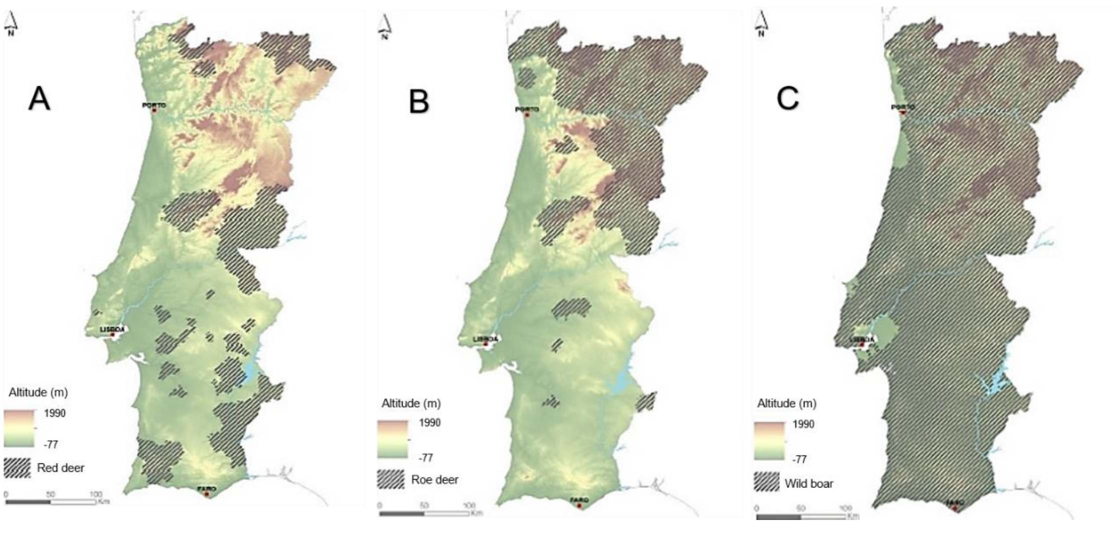


Figure 6 – Current distribution of red deer (A), roe deer (B) and wild boar (C) in Portugal. Source: Álvares *et al*., 2015.

There also have been changes in the use of measures to prevent and minimize damages on livestock, which are known to influence the level of wolf predation (Vos, 2000; Imbert *et al*., 2016; Pimenta *et al*., 2017). Several conservation programs conducted during the last few decades in Portugal have been promoting the use of electric fences and especially of livestock guarding dogs, to effectively protect livestock from wolf attacks (Ribeiro and Petrucci-Fonseca, 2004; Álvares *et al*., 2015). These projects make use of the four recognized breeds of livestock guarding dogs autochthonous from Portugal, which are simultaneously a cultural and biological legacy (Álvares and Primavera, 2004), and have already provided several hundreds of dog to livestock breeders under careful management to efficiently protect domestic animals from predators (Ribeiro and Petrucci-Fonseca, 2004). These projects promoting livestock guarding dogs have targeted, in particular, the Montesinho/Bragança region, which along with the expansion of wild ungulates, is often considered as the main responsible for the current low numbers of wolf attacks to livestock in this area (Álvares *et al*., 2015). On contrary, in Peneda-Gerês region, livestock, particularly cattle, has been increasingly managed in an extensive and unprotected way, with poor vigilance, which seems to expose livestock to higher risk of wolf predation (Álvares *et al*., 2014; Pimenta *et al*., 2017). Considering all these recent socio-ecological changes on Portuguese wolf range, the updated knowledge on wolf trophic ecology, including diet, prey selection and feeding behaviour, would be crucial to evaluate current level of wolf predation on livestock and their spatial-temporal variation, a topic with important management implications. In fact, despite several existing studies regarding wolf feeding habits in Portugal, all based in scat analysis with no genetic validation, there are no recent information on wolf diet as most studies were conducted during late 1990s and early 2000s (with the exception of Torres *et al*., 2015a). Furthermore, there is still a lack of knowledge regarding prey selection, kill rates and feeding behaviour (e.g. predation vs scavenging) of this carnivore, particularly focusing domestic prey species. For better comprise wolf trophic ecology in human dominated landscapes, it is crucial to understand the predator-prey systems, not only in an ecological context, but also in an economic and management perspective (Hosseini-Zavarei *et al*., 2013; Zlatanova *et al*., 2014). In this context, is relevant to evaluate the influence of livestock husbandry practices and wild prey availability in wolf feeding ecology, considering spatial, temporal and even individual patterns of variation (Pimenta *et al*., 2017). As recently recognized in the Wolf Action Plan for Portugal, still waiting official approval (Álvares *et al*., 2015), updating the current knowledge of all these topics is of great importance as they will help to prevent and minimize the long term conflict so intrinsic in the history between Humans and Wolves.

SCOPE AND GOALS OF THIS STUDY

This study aims to address wolf trophic ecology, such as diet, prey selection and feeding behaviour, in a multi-prey system dominated by domestic animals, using Portuguese wolves as a model. For this propose, information will be obtained in three different areas of Portugal (Montesinho, Sul do Douro and Peneda-Gerês), based in the analysis of scats genetically identified, allowing to access regional, temporal and individual patterns of wolf feeding habits. Regional patterns will be inferred from the specify conditions of each study area regarding livestock husbandry practices and wild prey availability, while temporal patterns will be evaluated by comparing results obtained during the last decades in previous studies for the same areas. Regarding individual patterns of feeding habits, a topic poorly studied worldwide, this study will conduct a preliminary approach based on scats of individual wolves genetically identified in order to evaluate dietary differences in several individuals belonging to a same pack and in the same individual during a long period of time.

In particular, the main goals of this study are to:

- Assess wolf diet, prey selection and seasonal variation in three areas with different ecological conditions, based in the analysis of scats genetically validated. Evaluate temporal variation on wolf feeding habits, and particularly on the ratio between domestic and wild prey consumption, based in available data from previous years;
- Infer wolf feeding strategy (predation vs scavenging) in each study area, based on the comparison between prey occurrences in scat content and livestock predation rates declared to National authorities;
- Determine individual differences on feeding behaviour of wolves concerning intra-pack individual variation and individual variation along time.

The proposed goals are expected to provide valuable insights on wolf trophic ecology in humanized landscapes, with worldwide implications, and also to update the knowledge on wolf feeding habits in Portugal. Hopefully, this work will help to adopt better management strategies for reducing the impact of wolves on livestock, and consequently, the human-wolf conflict, with the purpose of maintaining or recovering wolf populations both in Portugal and worldwide.

CHAPTER II

STUDY AREAS

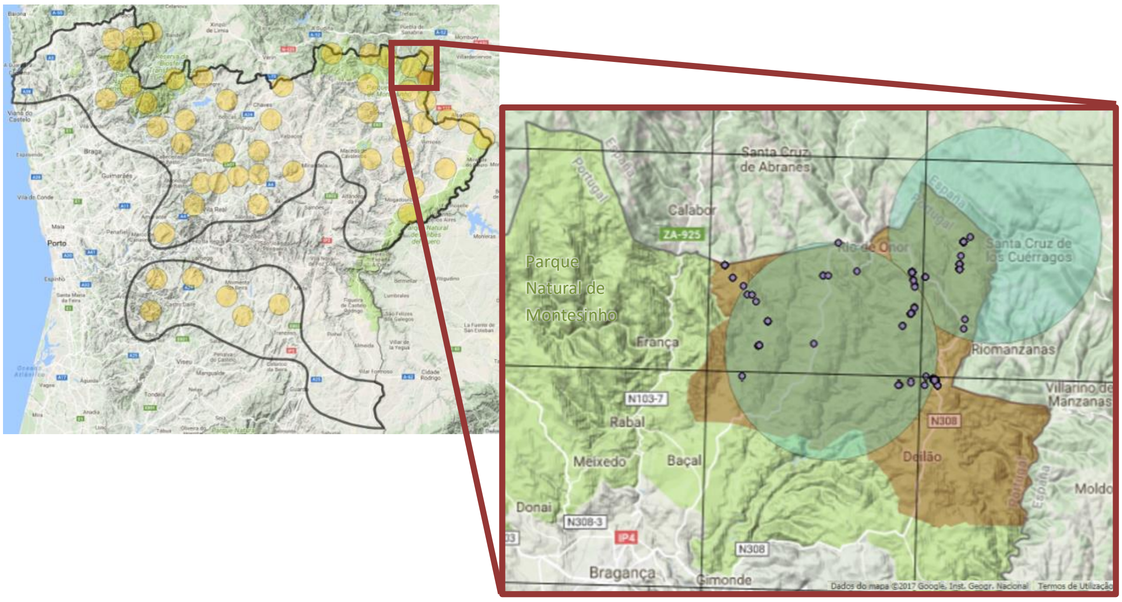
This study was conducted in three distinct areas within wolf range in Portugal, each of them comprising territories of known packs (Pimenta *et al*., 2005):

- Montesinho – comprising the approximate territories of Rachas and Minas packs, located in Bragança (NE Portugal);
- Sul do Douro – comprising the approximate territory of Leomil pack, located in Moimenta da Beira (Central Portugal);
- Peneda-Gerês – comprising the approximate territories of Soajo and Vez packs, located in Arcos de Valdevez (NW Portugal).

Each study area has different ecological conditions, regarding livestock species and husbandry systems, as well as wild prey diversity and abundance. Following, is presented a general description of each study area in relation to: administrative regions (e.g. Parishes or “Freguesias”, used for study delimitation); wolf packs; livestock species and numbers; main livestock husbandry methods; wild prey diversity and abundance; and previous studies on wolf diet, used to address temporal patterns. Study areas were delimited in order to coincide with regions or packs already targeted for dietary studies in previous years, and where there is available information on mostly grey literature, such as unpublished academic thesis.

MONTESINHO – RACHAS/MINAS PACKS

Montesinho study area is located in the Northeast of Montesinho Natural Park, comprising the areas of three parishes belonging to Bragança county, with a total area of 148.33 km^2 (Figure 7 and Table 1). This area includes the approximate territories of two wolf packs – known as Rachas and Minas – considered as reproductively stable (Pimenta *et al*., 2005). This study area has been the target of several studies addressing wolf diet conducted in late 1980s (Petrucci-Fonseca, 1990) in 1990/1992 (Moreira, 1992) and in 1996/1997 (Pimenta, 1998). The general description of this area concerning livestock and wild prey availability is presented in Table 1 (see Appendix I).



- Wolf distribution range
- Confirmed Wolf packs (Pimenta *et al.*, 2005)
- Montesinho study area
- Montesinho Natural Park
- Study area delimitation - UTM 10x10 Km
- Approx. territory of Rachas and Minas packs
- Localization of scats collected for this study

Figure 7 – Localization of "Montesinho" study area in relation to wolf range and known packs in Portugal (according to Pimenta *et al*., 2005) and inset map with the study area delimitation and location of scats collected and analysed in this study.

Table 1 – Characterization of Montesinho study area (comprising Aveleda, Deilão and Rio de Onor parishes), regarding wild and domestic prey availability based on available information, namely livestock species and numbers, main livestock husbandry methods, and wild prey diversity and abundance.

		Source
Study area delimitation	**Counties:** Bragança. **Parishes:** Aveleda, Deilão and Rio de Onor.	
Domestic prey species and numbers	Cattle (218 individuals in 2009), pig (96 individuals in 2009), sheep (1503 individuals in 2009), goats (127 individuals in 2009), horses (68 individuals in 2009), poultry (1900 individuals in 2009) and rabbits (341 individuals in 2009).	INE, 2011
Livestock husbandry system	Extensive grazing of small ruminants only during day time and always guarded with shepherd and several livestock guarding dogs. Rabbit production in intensive farms.	Pimenta, 1998; personal observation
Wild ungulates species and abundance	Red deer, roe deer and wild boar. Few information on abundance, but appears to be high for all these species. Red deer occurs at 3.1 ind./100 ha and roe deer occurs at 4.9 ind./100 ha. The available information on the abundance of wild boar is correspondent to all area of Portugal, occurring estimated to 10 ind./100 ha.	CONFAGRI, 2009; Valente *et al*., 2014; Santos 2015; Terras de Sicó, 2017

Sul do Douro – Leomil Pack

Sul do Douro study area is located in Central Portugal, comprising the areas of 12 parishes belonging to the counties of Vila Nova de Paiva and Moimenta da Beira, with a total area of 229.28 km^2 (Figure 8 and Table 2). This area includes the approximate territory of the Leomil pack. The reproduction of this pack was considered probable, with evidences that may support that possibility (e.g. collected information from the local population, howls from wolf cubs) (Pimenta *et al*., 2005). This study area has been the target of studies conducted in the first years of 2000 (Alexandre *et al*., 2000; Quaresma, 2002; Sobral, 2006; Pinto, 2008). The general description of this area concerning livestock and wild prey availability is presented in Table 2 (see Appendix I).

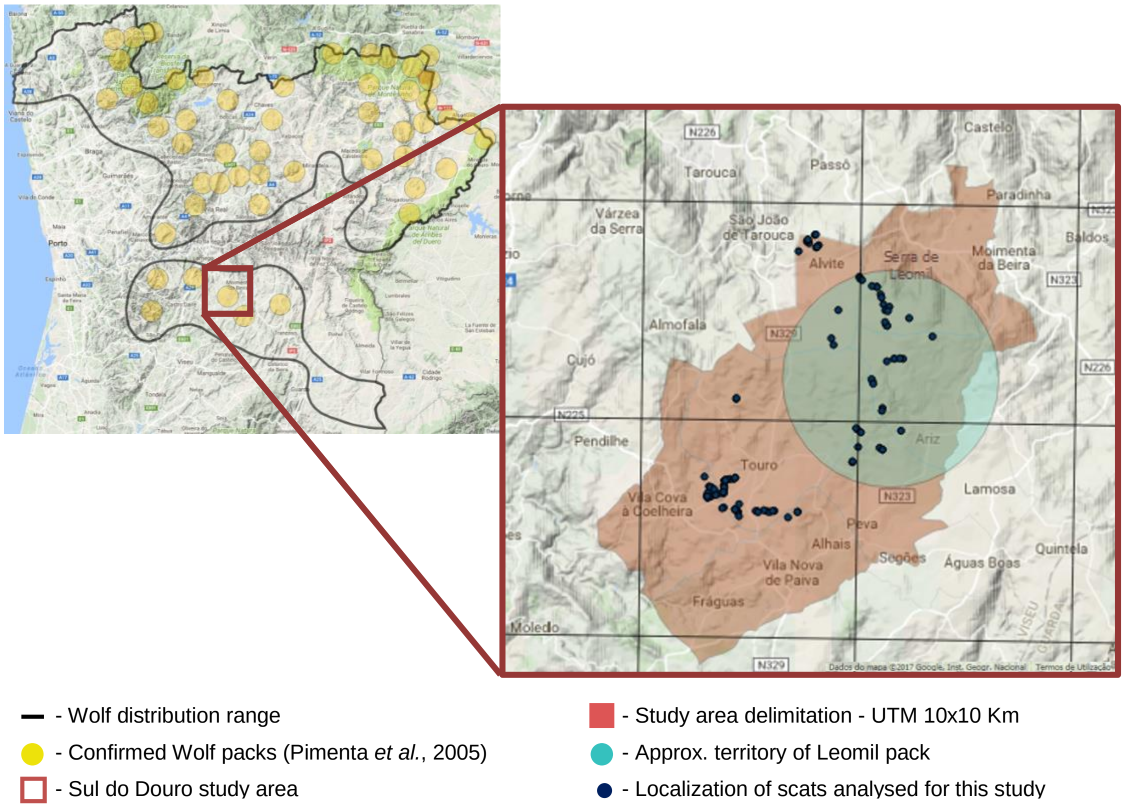


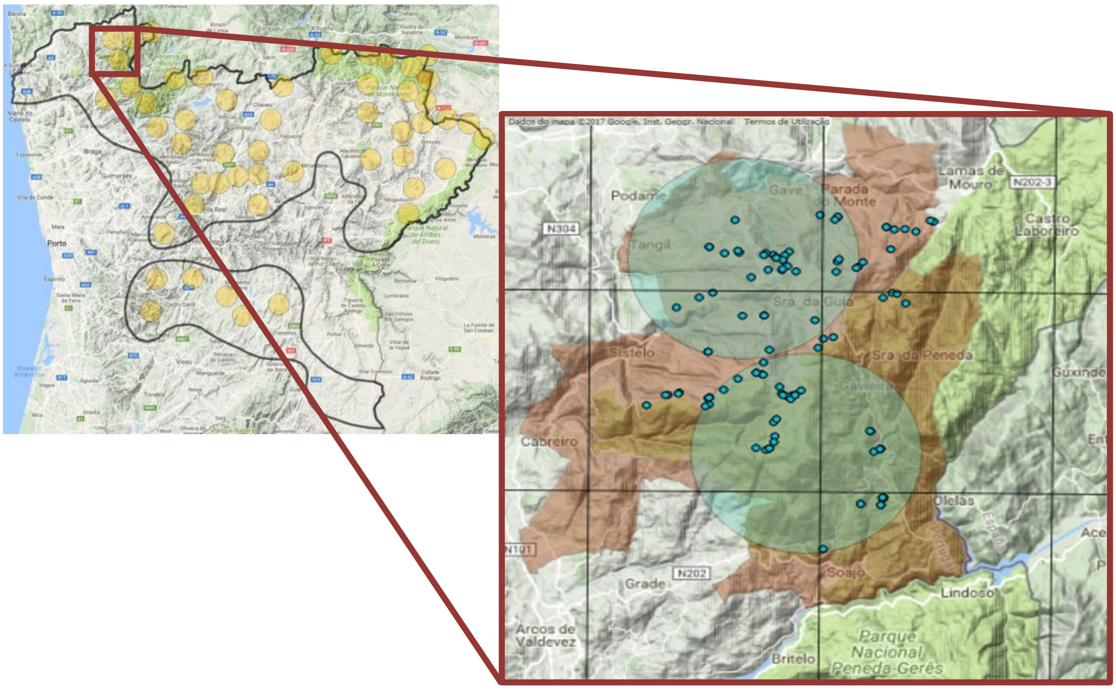
Figure 8 – Localization of Sul do Douro study area in relation to wolf range and known packs in Portugal (according to Pimenta *et al*., 2005) and inset map with the study area delimitation and location of scats analysed from this area.

Table 2 – Characterization of the Sul do Douro study area (comprising Aldeia de Nacomba, Alhais, Alvite, Ariz, Fráguas, Leomil, Pêra Velha, Segões, Touro, Vila Cova à Coelheira and Vila Nova de Paiva parishes), regarding wild and domestic prey availability based on available information, namely livestock species and numbers, main livestock husbandry methods, and wild prey diversity and abundance.

		Source
Study area delimitation	**Counties:** Vila Nova de Paiva and Moimenta da Beira. **Parishes:** Aldeia de Nacomba, Alhais, Alvite, Ariz, Fráguas, Leomil, Pêra Velha, Peva, Segões, Touro, Vila Cova à Coelheira and Vila Nova de Paiva.	
Domestic prey species and numbers	Cattle (2298 individuals in 2009), pig (698 individuals in 2009), sheep (1983 individuals in 2009), goats (1978 individuals in 2009), horses (122 individuals in 2009), poultry (347974 individuals in 2009) and rabbits (16439 individuals in 2009).	INE, 2011
Livestock husbandry system	Extensive grazing of small ruminants (sheep and goats) during the day time, usually with the presence of a shepherd and/or mid-size dogs. Intensive farms of rabbit, pigs and poultry.	Torres *et al*., 2015a; Personal observations
Wild ungulates species and abundance	Wild boar. There is no published information available on abundances, but according to CONFAGRI (2009) and Terras de Sicó (2017), that present wild boar abundance for all area of Portugal, seems to occur in high densities (estimated 10 ind./ 100 ha).	CONFAGRI, 2009; Terras de Sicó, 2017

PENEDA-GERÊS – SOAJO/VEZ PACKS

The Peneda-Gerês study area is located in the Northwest Portugal, comprising the areas of 10 parishes belonging to the counties of Arcos de Valdevez, Melgaço and Monção, with a total area of 304.14 km^2 (Figure 9 and Table 3). This area includes the approximate territories of two wolf packs – known as Soajo and Vez – considered as reproductively stable (Pimenta *et al*., 2005). This study area has been target of several studies addressing wolf diet, conducted in late 1980s (Petrucci-Fonseca, 1990), late 1990s (Lançós, 1999), and in the first decade of 2000 (Guerra, 2004; Álvares, 2011). The general description of this area concerning livestock and wild prey availability is presented in Table 1 (see Appendix I).



- Wolf distribution range
- Confirmed Wolf packs (Pimenta *et al.*, 2005)
- Peneda-Gerês study area
- Peneda-Gerês National Park
- Study area delimitation - UTM 10x10 Km
- Approx. territory of Vez and Soajo packs
- Localization of scats analysed for this study

Figure 9 – Localization of Peneda-Gerês study area in relation to wolf range and known pack in Portugal (according with Pimenta *et al*., 2005) and inset map with the study area delimitation and location of scats analysed for this area.

Table 3 – Characterization of the Peneda-Gerês study area (comprising Cabreiro, Cubalhão, Gave, Gavieira, Gondoriz, Parada de Monte, Riba de Mouro, Sistelo, Soajo and Tangil parishes), regarding wild and domestic prey availability based on available information, namely livestock species and numbers, main livestock husbandry methods and wild prey diversity and abundance.

		Source
Study area delimitation	**Counties:** Arcos de Valdevez, Melgaço and Monção. **Parishes:** Cabreiro, Cubalhão, Gave, Gavieira, Gondoriz, Parada de Monte, Riba de Mouro, Sistelo, Soajo and Tangil.	
Domestic prey species and numbers	Cattle (4727 individuals in 2009), pig (148 individuals in 2009), sheep (4611 individuals in 2009), goats (1120 individuals in 2009), horses (1144 individuals in 2009), poultry (9822 individuals in 2009) and rabbits (2084 individuals in 2009).	INE, 2011
Livestock husbandry system	Extensive grazing of goats and sheep with shepherd and/or guarding dogs. Cattle is free-ranging, especially during Summer with few or none vigilance. Horses are free-ranging all year round.	Álvares, 2011

Wild ungulates species and abundance	Roe deer and wild boar. Few available information on abundance of these two species, but according to CONFAGRI (2009) and Terras de Sicó (2017) wild boar seems to occur in high densities in all Portugal (estimated 10 ind./100 ha), while roe deer should occur at low densities (estimated in 1.6 ind/100 ha for other areas of Peneda-Gerês National Park). Recently, there have been occasional sightings of wild goat in this area.	Ferreira, 2003; Moço *et al*, 2006; CONFAGRI, 2009; Terras de Sicó, 2017; F.Álvares, pers. observations

CHAPTER III

METHODOLOGY

Several methods allow the study of wolf feeding ecology, such as direct observation of feeding animals, detection of prey remains, analysis of gastrointestinal contents and scat analysis (Peterson and Ciucci, 2003; Klare *et al*., 2011). In relation with the other methods, the scat analysis reveals to be the most efficient and widely used, considering that is a non-invasive approach, allowing large sample sizes, even in populations with low density (Ciucci *et al*, 1996; Klare *et al*., 2011). Taking into account the advantages of this method, and in order to compare the results from this work with previous studies also based in the same methodological approach, for this study were used analysis of scats genetically confirmed to determine wolf feeding habits.

FIELD SAMPLING AND SAMPLE VALIDATION

Scats were sampled, in each study area, along transects in travel routs used by wolves (trails and unpaved roads), particularly in places or land marks frequently used to deposit faeces for territorial scent-marking, such as crossroads (Barja *et al*., 2005). The transects were made by vehicle at low speed (<10 km/h) to allow the visibility of potential scats, while in each crossroad and approximately 100 m for each side, sampling was made on foot. To identify scats in the field and avoid collecting samples from other canid species (such as red fox and domestic dog), it was taken in consideration different criteria, namely the physical aspect (shape and size), scent and composition of scats (Sanz and Domínguez, 2015), the proximity to known core areas of breeding packs (Pimenta *et al*., 2005) and the level of human presence or activity to decrease the possibility to find scats from domestic dogs. In case of doubt regarding taxonomic affiliation, the scat was ignored.

Each scat attributed to wolf was collected, stored in DNA-free tubes labelled with an ID and preserved in 96% ethanol. A small sample from each scat was used for posterior genetic analysis performed in CIBIO's (Research Centre of Biodiversity and Genetic Resources, University of Porto) lab for species and individual molecular identification. Species identification was assessed through the amplification of an approximately 425 bp sized fragmented of the mtDNA control region, and then samples with wolf mtDNA were genotyped for a set of 13 microsatellites for individual identification (for further details on the molecular procedures for scat analysis see: Nakamura *et al*., 2017). The precise location of each collected scat was registered with the application MapIt (version 5.0.5), a tool developed for the collection of data for Geographic Information Systems (GIS), based on Global Positioning Systems (GPS).

Scat samples for the study areas of Peneda-Gerês (N=118) and Sul do Douro (N=91) were already available and genetically analysed in the scope of on-going monitoring projects conducted by CIBIO, being collected in previous years: 2008 to 2010 for Peneda-Gerês; 2011 to 2013 for Sul do Douro. Field sampling for Montesinho study area was conducted in the scope of this study, during four field trips of 3 to 4 days duration each, comprising Summer 2016, Autumn 2016, Winter 2017 and Spring 2017. A total of 164 scats were collected in Montesinho, from which 14 (8%) were genetically confirmed as being from domestic dog, 31 (19%) as being from red fox, 21 (13%) were it was not possible to genetically identify any species, and 98 (60%) were genetically confirmed as being from wolves and, consequently, used for posterior dietary analysis.

SAMPLE SELECTION AND TRIAL

Scat samples already available for the study areas of Sul do Douro and Peneda-Gerês were selected according several procedures, based in a detailed evaluation of respective information stored at CIBIO's database (comprising samples collected since 2006).

Regarding the time period of sampling, scats collected along several years were selected in order to fulfil the following requirements: sample size >90 to allow reliable results; spatial representativeness of the target pack or packs; and a uniform distribution throughout seasons.

Regarding sample selection to address individual variation of wolf feeding ecology, it was considered some criteria on scat samples with individual molecular identification. For the intra-pack analysis to evaluate dietary variation among members of the same pack, it was considered that samples should contain at least three different individuals assigned to the same pack, each with a minimum of two recaptures in scats, during a short period of time (maximum of one month) to avoid a temporal bias. Sampling from Peneda-Gerês had the ideal situation, considered that the 14 selected scats from 4 different individuals were all collected in the same day. For intra-individual analysis to evaluate diet variation in a single wolf along time, it was considered that samples from the same wolf should have a large number of recaptures in scats, during the maximum time period possible, enabling reliable results. Sampling from Sul do Douro had the ideal situation considering that the 2 selected individual have more than 10 recaptures each, during a minimum period of 4 years.

Available scat samples from Sul do Douro and Peneda-Gerês were constituted by a small terminal portion of the whole scat (approximately 25% of the original scat volume), collected for genetic validation. Before conducting analysis of these scats to assess diet, was performed a trial with the goal of verifying if information on prey items could be lost when just analysing a small proportion of the scat instead of the whole scat.
The samples from Sul do Douro and Peneda-Gerês were not possible to include in this trial considering that the available material was only the proportion used for genetic validation, not allowing a final comparison with the remain scat. For this reason, the trial was conducted by using two datasets: 33 entire wolf scats previously collected during 2005 in Alto Minho region (NW Portugal) and without genetic validation (results not included in the main diet analysis conducted in this study), and 33 other entire scats collected in Montesinho in the scope of this study. For each scat was taken a portion of about 25% (equivalent to the one usually used for genetic validation and already available for Sul do Douro and Peneda-Gerês), which was analysed for prey item detection separately from the remain scat. The trial analysis revealed coherence in the results of 94% of all scats regarding number and type of detected prey in the main part of the scat vs in the small portion, with results being coincident in scats containing up to 3 different prey items (Appendix II). Differences between both portions were found in 3 scats from Montesinho dataset (9%) and only 1 scat from Alto Minho dataset (3%) (Appendix II). These results reflect a negligible role regarding wolf scat content when only a small portion of the scat was analysed, enabling trustworthy results of wolf diet based in samples usually collected for genetic validation, such as the ones available for Sul do Douro and Peneda-Gerês.

LABORATORY PROCEDURES

The collected scats genetically confirmed as being from wolves, were processed in laboratory to determine diet composition, using the point-frame method as a standardized procedure to identify hairs and other macroscopic remains of prey items (e.g Ciucci *et al*., 1996; Ciucci *et al*., 2004; Álvares, 2011). Following description of Ciucci *et al.* (2004) and Chamrad and Box (1964) as a guide line for the construction of the point-frame, was used an aluminium box and a frame containing pins at a 90º angle through a wooden bar, fitted to slide along the top of the main box. Depending on their size, the scats ready for analysis were uniformly spread into squares of 10cm, 8cm or 5cm, assuming a random distribution of each item. The amount of pins used was accordingly to the size of the square. For example, for the 8 cm square, 64 pin drops, evenly spread, were used for the analysis of the faecal sample (8 drops x 8 fixed positions). Where each pin dropped, the correspondent item was recorded, and the hairs removed for further identification. This method was demonstrated to be reliable and efficient for wolf diet studies based on scat analysis as it is a trustworthy alternative to hand and non-systematic separation of the macro-components, allowing similar results, a significant reduction in time and effort in the processing faecal samples and a standardized detection of all food items (Ciucci *et al.*, 2004).

In order to prepare the scats for the point-frame, each was soaked in water to desegregate macro-components through washing in a sieve with a mesh size of 0,5 mm. The microscopic remains were discarded, considering that they originate from food items in the same proportion as the macroscopic fraction. The scats were then oven-dry at a mean temperature of 65 ºC, during 1 day. The remains larger than the mesh size, representative of the macro-components, were then uniformly spread over the bottom of the point-frame box for the triage and quantification of the percentage of each macroscopic remain. Individual macro-components were separated according the following categories: hair, bones, feathers, vegetal material, mineral material, insects, garbage (plastic and other human materials) and non-identified material. For the correct identification of hairs, a reference collection was elaborated, consisting in hairs collected from each potential wolf prey in the study areas. This reference collection is now available in CIBIO's laboratory for future studies. The hairs selected in each scat were the basis for identifying the consumed prey through the microscopic examination of their cuticular patter, medulla and cross-section. Based in the hair particularities of each prey species, the specific *taxon* was identified whenever possible, following the criteria from Debrot *et al.* (1982), Teerink (1991), Valente *et al*. (2015), De Marinis and Asprea (2006) and unpublished data by Sara Roque (Grupo Lobo/FLUP). Remains of feathers were identified microscopically to the taxonomic Order, comparing with material from the reference collection. The basis for their identification were the characteristic of the nodes and internodes, that are specific for the taxonomic Orders found in this study (unpublished data from Sara Roque, Grupo Lobo/FLUP). The items considered non-food material were identified macroscopically and their number of occurrences were quantified (Appendix III), although not included in the final results as prey class. These items consisted in wolf hairs, non-identified material, material ingested intentionally, such as bones and purgative plants, and finally, material considered to be ingested involuntarily, such as mineral material, plant leaves, insects and garbage from human origin.

DATA ON DOMESTIC AND WILD UNGULATES

To determine the availability of domestic and wild ungulates in each study area, as well as the level of livestock depredation by wolves, it was used information obtained from official statistics for livestock and published data for wild ungulates (Appendix I). Livestock numbers were obtained from the agricultural statistics of 'Recenceamentos Gerais da Agricultura', carried out in 2009 and retrieved at the level of parishes ("Freguesia") included in each study area (INE, 2011). Population sizes of wild ungulates were estimated from available information on population densities of each species, which was then extrapolated for the area comprised by all parishes included in each study area. Considering the general lack of regular population sizes from the nearest region or time period available, namely: densities of red deer and roe deer for Montesinho Natural Park (Valente *et al*., 2014; Santos, 2015); densities of roe deer for Peneda-Gerês National Park (Ferreira, 2003) and general mean abundance of wild boar estimated for all Portugal (CONFAGRI, 2009; Terras de Sicó, 2017).

To quantify the level of wolf predation on domestic ungulates in each study area, data was obtained from official statistics of wolf attacks to livestock claimed for compensation to ICNF, at the parish level. In order to ensure the right to compensation, each wolf kill was examined in the field by trained wildlife rangers from ICNF to confirm it has been predated by wolves. For each study area, it was considered the number of wolf attacks to each livestock species that were declared during the same time period covered by diet analysis.

DATA ANALYSIS

DIET AND PREY SELECTION

To evaluate wolf diet based in scat analysis, was used the quantification of prey in terms of Frequency of Occurrence (F.O) and Consumed Biomass (Biomass), considered the most common and informative approaches in wolf diet studies (Ciucci *et al*., 1996; Spaulding *et al*., 2000). Frequency of Occurrence is expressed as an absolute percentage of the number of occurrences of each prey item in relation with the total number of occurrences of all identified prey, allowing a direct knowledge of the relative importance of each prey item in the sample. In order to categorize the importance of F.O. for each prey item, values were classified according with Ruprecht (1979) in: basic food resource (F.O. ≥ 20%); regular food resource (20% > F.O. > 5%); supplementary food resource (5% ≥ F.O. > 1%); occasional food resource (F.O. ≤ 1%). Despite being a very informative method, F.O. can have some associated bias, considering it may overestimate large bodied or less consumed prey, that can be found in several different scats but originated from one single prey, and underestimate more commonly consumed prey, considering the differences that can exist in a ratio surface/volume between a small and a large prey (Ciucci *et al.*, 1996). Therefore, to complement the dietary analysis, was also calculated the Consumed Biomass.

The percentage of Biomass of each prey class was estimated using the model of Weaver (1993), adjusted from a previous model from Floyd *et al.* (1978), and is represented by a linear regression:

$$y = 0.439 + 0.008x$$

where the dependent variable (y) represent the biomass ingested per collected scat, and the independent variable (x) represents the mean live weight (kg) of each prey class identified in the scat. Multiplying the value of y by the number of scats in which the corresponding prey was found, is possible to obtain the total amount of Biomass for each prey class. The identification of consumed adults and juveniles in this study was not possible to obtain for most prey species, with the exception of wild boar, which the physical and microscopic analyse of guarding hairs allowed to roughly distinguish their age class as adults and juveniles with less than 6 months (unpublished data from Sara Roque, Grupo Lobo/FLUP). For the other prey classes, were considered average weights of adult individuals from each prey class, obtained from available information and, whenever relevant (such as goats and sheep (*Ovis aries*)), adapted to the local breeds in each study area. In the identification of cat hairs, it was not possible to distinguish with certainty between wild cat (*Felis silvestris*) and domestic cat (*Felis catus*). For that reason, it was considered the mean weight between the two species (Appendix IV). Since all identified feathers were from Galliformes, was used an average weight from domestic chickens raised in intensive farms. To evaluate statistical differences of specific results (e.g. prey selection, seasonal variation) in each study area, was used a χ^2 test, with a significance level of 5% and Yates correction for 1 degree of freedom (Cochran, 1952). The Yates correction for continuity is applied in order to minimize the possible bias of the traditional restrictions with respect to minimum expected frequencies, dictating that the expected frequencies must be superior to 5 (Cochran, 1952; Roscoe and Byars, 1971).

The analysis of prey selection on domestic and wild prey was quantified through the selectivity Ivlev's Index (D), modified by Jacobs (1974) (Krebs, 1989 *in* Álvares, 2011):

$$D = \frac{(r - p)}{(r + p - 2 \cdot r \cdot p)}$$

being r the proportion of each prey class in wolf diet and p the proportion of that prey in terms of population size in the respective study area. Values of estimated population size for each prey species are described in Appendix I. The index varies between -1 (the complete avoidance of the species) and 1 (maximum positive selection). When equals 0 means that consumption is proportional to abundance.

OTHER DIET PARAMETERS

The diversity of wolf diet in each study area was calculated by Shannon's Diversity Index (H') (Pite and Avelar, 1996 *in* Guerra, 2004), according to:

$$H' = -\sum p_i \cdot ln(p_i)$$

being p_i the proportion of the prey class i in the diet. When all classes are equally represented in wolf diet, the diversity reaches the maximum value and assumes the value of $ln(S)$, being S the total number of prey classes. Assuming that the specific diversity translates simultaneously the number of prey and their representativeness in wolf diet, the final value can be the same for different diets. Therefore, the equitability is also estimated (H'/H_{max}), tending to 0 when almost all diet is constituted by a single species, and tends to 1 when prey classes are equally represented.

Niche breadth (N.B.) was calculated with Standardized Levin's Index (Krebs, 1989 *in* Álvares, 2011), according to:

$$N.B. = \frac{(\sum p_i^2)^{-1} - 1}{(N-1)}$$

being N the number of prey classes identified and p_i the proportion of each prey class in the diet. The index varied between 0 and 1, being 0 when only one prey class is being explored, and 1 when all prey classes are exploited equally. This index reflects the degree of specialization of the diet.

Trophic overlapping in the use of different prey species between study areas and seasons was quantified by the analysis of Niche Overlap (N.O.), calculated with Pianka's Symmetrical Index (Krebs, 1989 *in* Álvares, 2011):

$$N.O. = \frac{\sum(p_{ij} \cdot p_{ik})}{\sqrt{(\sum p_{ij}^2 \cdot \sum p_{ik}^2)}}$$

being p the proportion of a certain prey class i in the season j and k. The overlap reaches the maximum value of 1 if all prey classes are exploited equally during all season (or between study areas), and reaches the minimum value of 0 if there is no common prey along the seasons (or between study areas). Seasons were defined as: Winter (January 1^{st} to March 31^{th}); Spring (April 1^{st} to June 30^{th}); Summer (July 1^{st} to September 30^{th}) and Autumn (October 1^{st} to December 31^{th}).

To infer the wolf feeding behaviour related to livestock consumption in each study area (predation vs scavenging), was determined the ration between the Frequency of Occurrence of each livestock species in wolf diet and the respective number of wolf attacks declared in the same period. The impact of predation on domestic ungulates was based on declared attacks to livestock attributed to wolf, obtained from official data of ICNF.

To assess temporal variation on wolf feeding habits in each study area along the last decades, was made a comparison between the F.O. of the main prey classes (Wild ungulates, Domestic ungulates, Carnivores, Lagomorph and Others) obtained in this study and the ones obtained from available studies on wolf diet conducted in the same study areas in previous years. It is important to note that all previous studies on wolf diet were based in scats attributed to wolves, but not genetically confirmed. All spatial analysis were performed in a Geographic Information System using QGIS (version 2.18. Las Palmas), an open source of Quantum Geographic Information System.

Chapter IV

Results

I. Regional Patterns

1. Overall view

The study on wolf trophic ecology of the Iberian wolf was based on the analysis of a total of 307 scats genetically confirmed as being from wolves, distributed in three study areas: 98 (32%) in Montesinho, 91 (30%) in Sul do Douro and 118 (38%) in Peneda-Gerês. The global results of wolf diet expressed in F.O. revealed different feeding habits across three wolf population nuclei in Portugal (Figure 10).

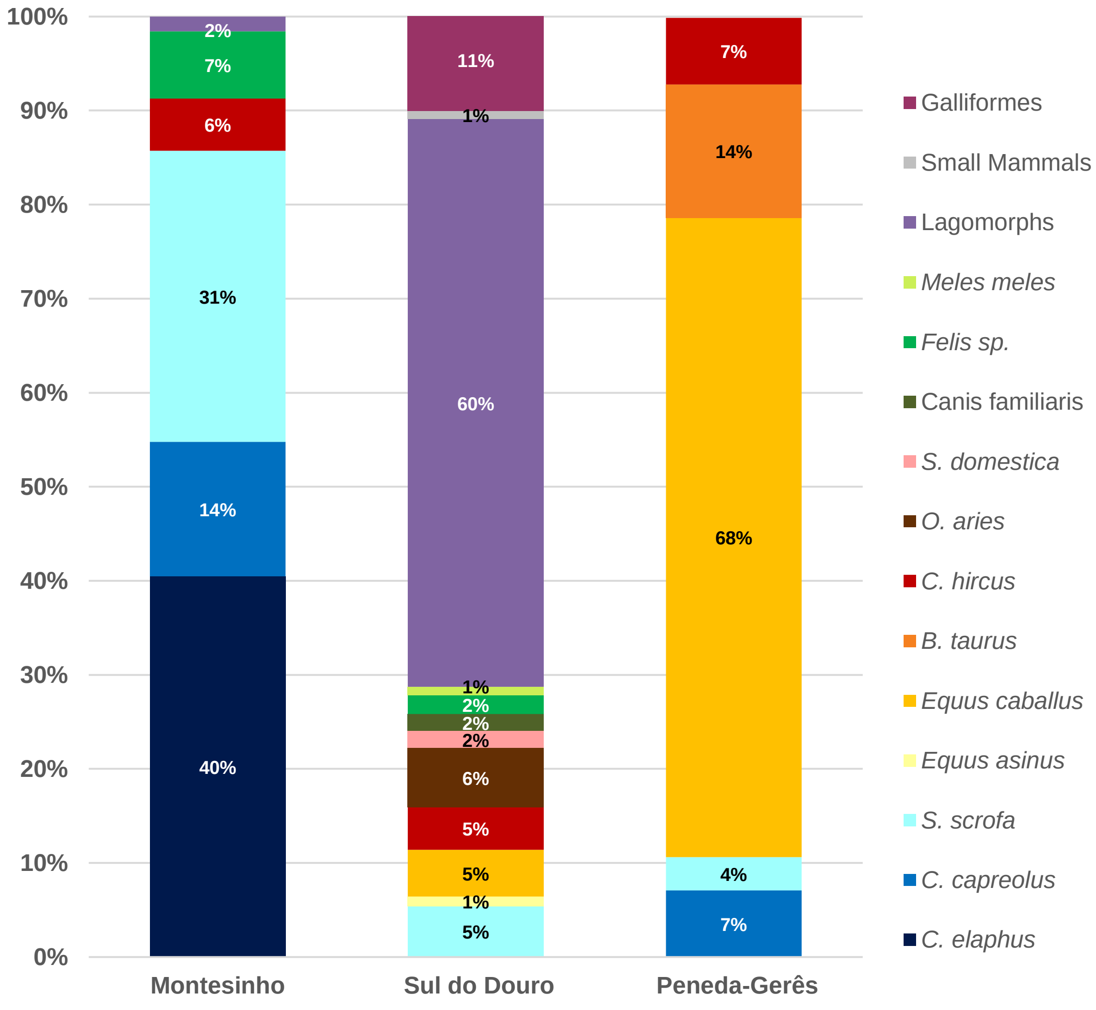


Figure 10 – Global results of wolf diet for the three study areas (Montesinho, Sul do Douro and Peneda-Gerês). Values are expressed in F.O.

In particular, there are evident differences between the consumption of domestic and wild prey, reflecting the diversity and availability of prey species in each area. In

Montesinho, three species of wild ungulates are the most consumed prey (85% F.O.). In Sul do Douro, wolf diet is mostly consisted by Lagomorphs (60% F.O.), while in Peneda-Gerês domestic ungulates, mainly horses, represent the majority of consumed prey, with 89% F.O.

Sul do Douro has the highest values of diet diversity (H'=2.28) with 12 prey items detected, but the lowest value of niche breadth (N.B.=0.140) (Figure 11), suggesting some specialization in only one prey class (e.g. Lagomorphs). Montesinho, with 6 prey items detected, showed a more even exploitation of all prey classes, while Peneda-Gerês, with 5 prey items detected, showed also some diet specialization, particularly on horses. Overlap of trophic niches between study areas presented low values (see Appendix V), confirming the huge regional differences on wolf diet within the Portuguese range of this carnivore. In the following chapters, is presented a more detailed analysis of wolf trophic ecology in each of the study areas.

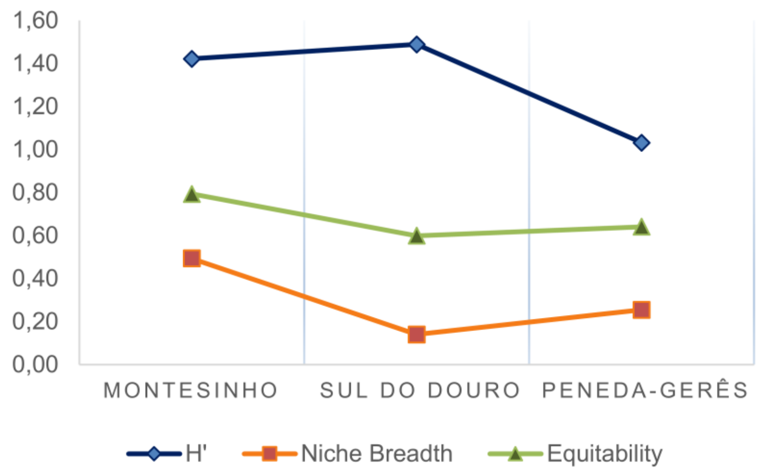


Figure 11 – Diet diversity (H' and Equitability – Shannon-Wever Index) and Niche Breadth (modified Simpson Index) of wolf diet in three study areas: Montesinho, Sul do Douro and Peneda-Gerês.

2. Montesinho - Rachas/Minas Packs

2.1 Feeding habits

In Montesinho (comprising Rachas and Minas packs), the majority of the 98 scats that were analysed presented one prey (72%), while in 26% were identified two prey and only in 2% of the scats were found three different prey species. Prey classes identified in Montesinho study area included five species – four ungulates and one carnivore – and one order – Lagomorphs (Table 4). Regarding non-food material found in wolf scats from Montesinho study area, they consisted in wolf hairs, bone material, purgative plants, mineral materials, plant leaves, insects and garbage from with human origin (see Appendix III).

Within the 6 identified prey classes, three wild ungulates are the dominant category, representing the majority of the Biomass (92%), while livestock is only represented by one species – domestic goat – comprising only 4% of the consumed Biomass (Table 4). Red deer (40% F.O) and adult wild boar (23% F.O.) stand out for being the only basic resources, followed by roe deer as regular resource (14% F.O.). Other regular resources, although with much less expression, were cat and goat, represented by 7% and 6% of F.O., respectively. As a supplementary resource, the Lagomorphs were found in only two scats, always associated with larger prey, representing 2% of F.O (Table 4).

Table 4 – Wolf diet in Montesinho study area (N=98 scats collected during 2016/2017), expressed in identified prey items, number of prey detections, Frequency of Occurrence, with correspondent classification of resources type, and Consumed Biomass.

		N	F.O. (%)	Resource	Biomass (%)
Wild Ungulates		**108**	**85**	**-**	**91**
C. elaphus		51	40	Basic	53
C. capreolus		18	14	Regular	9
S. scrofa	Adult	29	23	Basic	24
	Juvenile	10	8	Regular	5
Domestic Ungulates		**7**	**6**	**-**	**4**
C. hircus		7	6	Regular	4
Carnivores		**9**	**7**	**-**	**4**
Felis sp.		9	7	Regular	4
Lagomorphs		**2**	**2**	**Supplementary**	**1**
Total		**126**	**100**	**-**	**100**

2.2 Seasonal variation

Seasonal variation of wolf diet in Montesinho study area expressed in F.O. showed that wild ungulates are the main source of food during all seasons, with values ranging from 62% in Summer and 95% in Spring, although the relevance of each prey species varies greatly across seasons (Figure 12; Appendix VI). Most prey species are detected in all seasons, except Lagomorphs (only in Winter and Spring) and carnivores (except Spring). Red deer was the most consumed prey in most seasons, particularly in Summer (58% F.O.), except in Winter when wild boar is the most represented prey (48% F.O.). The analysis of the effect of seasonality revelled statistically significant differences for the consumption of red deer only between Winter and Summer (χ^2 = 4.29, 1 *g.l.*, $p < 0.05$) and for the consumption of roe deer between Spring and Summer (χ^2 = 5.34, 1 *g.l.*, $p < 0.05$). Wild boar presented significant differences between Winter and Summer (χ^2 = 12.64, *1 g.l.*, $p < 0.05$), between Spring and Summer (χ^2 = 4.84, 1 *g.l.*, $p < 0.05$) and between Summer and Autumn (χ^2 = 4.88, 1 *g.l.*, $p < 0.05$). As for cat, the significant differences were obtained between Winter and Summer (χ^2 = 5.72, 1 *g.l.*, $p < 0.05$) and between Spring and Summer (χ^2 = 7.15, *1 g.l.*, $p < 0.05$). The other prey species did not present any significant differences among seasons.

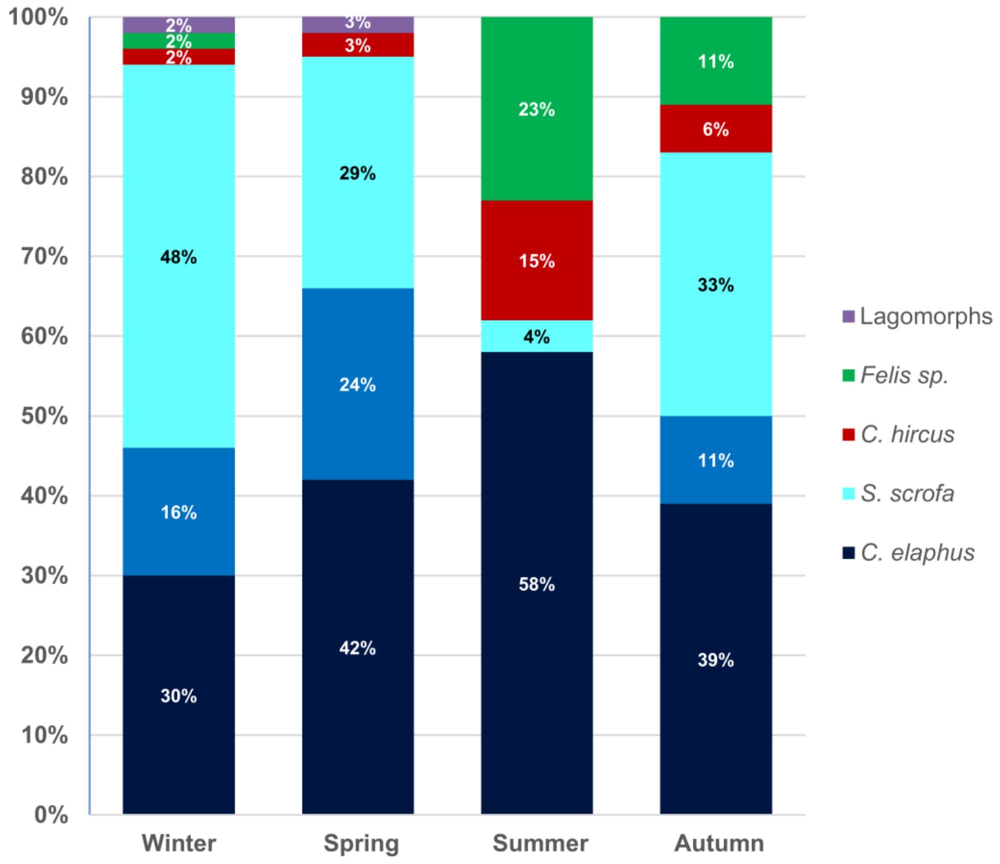


Figure 12 – Seasonal variation of wolf diet in Montesinho study area. Values expressed as F.O. of 98 scats collected in 2016/2017.

Regarding diet diversity and niche breadth, Autumn holds the highest values (H'=1,38; N.B.=0,612), while Summer presents the lowest values of diet diversity (H'=1,07) and Winter the lowest of niche breadth (N.B.=0,385) (Figure 13). However, no significant differences were detected among seasons for these two indexes (χ^2 teste; p>0.05 for all values).

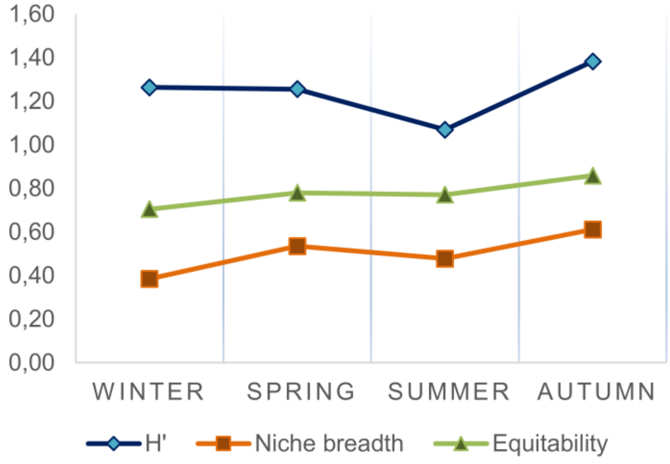


Figure 13 – Seasonal analysis of diet diversity (H' and Equitability – Shannon-Wever Index) and Niche Breadth (modified Simpson Index) for wolf diet in Montesinho study area.

Considering overlap of trophic niche, the comparison between Summer and Winter holds the lowest value (N.O.=0.527), revealing a more distinct exploitation of prey classes (Appendix VII).

2.3 PREY SELECTION AND FEEDING STRATEGY

Prey selection measured by Ivlev's Index shows a strong preference towards red deer and, at less extended, towards domestic goats despite their low F.O. in wolf diet and low availability in this study area (Figure 14). For the other two species of wild ungulates, wild boar and roe deer, together with Lagomorphs, wolves showed a negative selection. However, differences between the availability of prey and their

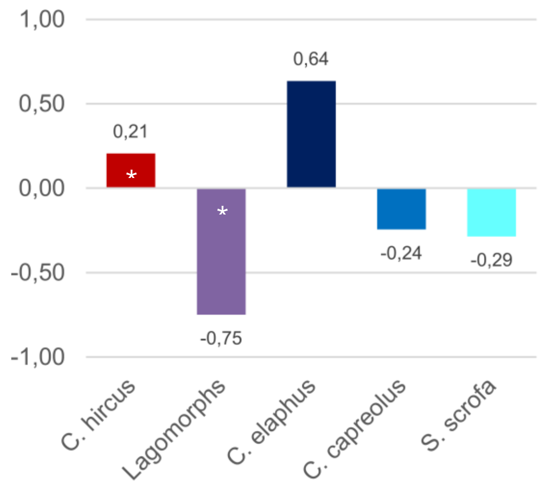


Figure 14 – Prey selection (Ivlev's Index) in Montesinho, according with the availability of main prey in the study area and F.O. in wolf diet. The index varies between -1 (complete rejection of a species) and 1 (maximum positive selection).
* Species with significant differences between occurrence in wolf diet and availability.

consumption reveal to be statistically significant only for goats and lagomorphs (χ^2 test, $p<0.05$).

The available data on wolf attacks to domestic ungulates in Montesinho study area, during the same period of diet sampling (January 2016 to February 2017), did not match with the results obtained in diet analysis (Figure 15; Appendix VIII). In fact, the only livestock attacks declared to ICNF in Portuguese parishes included in this study area correspond to 2 attacks to sheep, while scat analysis revealed only the presence of goats in wolf diet.

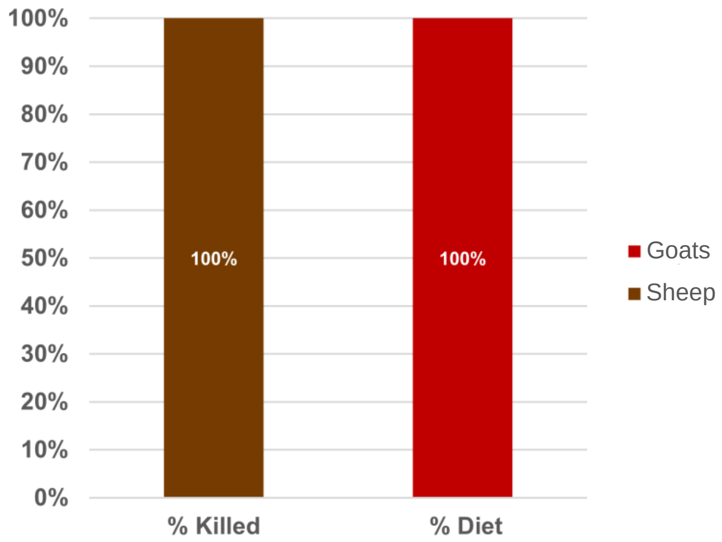


Figure 15 – Percentage of wolf attacks to each livestock species declared to ICNF in the parishes included in Montesinho study area (% Killed) in comparison with the percentage of prey detections in wolf scats (% Diet).

2.4 Temporal variation

Based on the results obtained in this study and in previous diet assessments in Montesinho study area, it becomes evident a steady and sharp increase in the consumption of wild ungulates along the last few decades following a decrease in the relevance of livestock in wolf diet (Figure 16). Almost two decades ago, wild ungulates only represented 29% of wolf diet in Montesinho study area, and currently they represent the majority of wolf diet (86% F.O.). On contrary, domestic ungulates were the main prey in 1990 (44%) while currently they constitute a minor portion of wolf diet (6% F.O.). Carnivores and Lagomorphs were always present in wolf diet as a regular and supplementary food resource, respectively, while other prey items, such as small mammals and birds, seem to be more frequent in the past and currently absent (Figure 16).

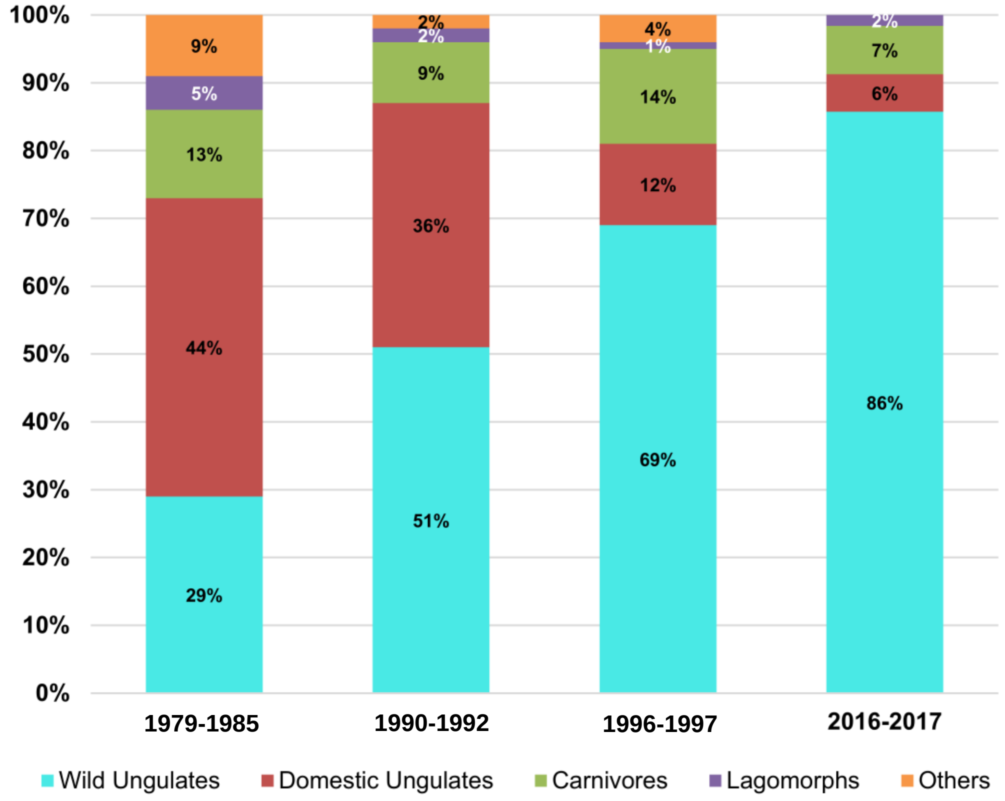


Figure 16 – Temporal variation of wolf diet along several years in Montesinho study area (Rachas/Minas packs). Values expressed in F.O. were obtain from: Petrucci-Fonseca (1990); Moreira (1992); Pimenta (1998); This study (2016-2017). The category "Others" includes Small Mammals and Galliformes.

3. Sul do Douro - Leomil Pack

3.1 Feeding habits

In Sul do Douro (comprising Leomil pack), 91 scats genetically attributed to wolf were collected between 2011 and 2013. The majority of the analysed scats presented one prey (76%), while in 21% were identified two prey, in 1% were found four prey and 2% of the scats presented only vegetable material. Prey classes identified in Sul do Douro study area included nine species – six ungulates and three carnivores – two orders – Lagomorphs and Galliformes – and the group of Small Mammals (Table 5). Regarding non-food items found in wolf scats from Sul do Douro study area, they included wolf hairs, non-identified material, bone material, purgative plants, mineral materials, plant leaves, insects and garbage from human origin (see Appendix III).

Within the identified prey classes, lagomorphs are the dominant category, representing 46% of the Biomass. Domestic ungulates represent 34% of the Biomass, while wild ungulates, represented only by one species (wild boar), and Galliformes represent 8%, and the remaining categories (Carnivores and Small Mammals) comprise only 5% of the consumed Biomass (Table 5).

Table 5 – Wolf diet in Sul do Douro study area (N=91 scats collected between 2011 and 2013), expressed in identified prey items, number of prey detections, Frequency of Occurrence, with correspondent classification of resource type, and consumed Biomass

		N	F.O. (%)	Resource	Biomass (%)
Wild Ungulates		**6**	**5**	**-**	**8**
S. scrofa	Adult	4	3	Supplementary	2
	Juvenile	2	2	Supplementary	6
Domestic Ungulates		**20**	**18**	**-**	**34**
E. caballus		5	5	Supplementary	15
E. asinus		1	1	Occasional	3
C. hircus		5	5	Supplementary	5
O. aries		7	6	Regular	6
S. domestica		2	2	Supplementary	5
Carnivores		**5**	**5**	**-**	**4**
C. familiaris		2	2	Supplementary	2
Felis sp.		2	2	Supplementary	1
M. meles		1	1	Occasional	1
Lagomorphs		**67**	**60**	**Basic**	**56**
Small Mammals		**1**	**1**	**Occasional**	**1**
Galliformes		**12**	**11**	**Regular**	**8**
Total		**111**	**100**	**-**	**100**

Lagomorphs (60% F.O.) stand out for being the only basic resource. To be notice that the presence of Lagomorphs in wolf scats from Sul do Douro occurred as the only consumed prey in 58% (N=53) of the scats, and in 15% (N=14) occurred associated with other prey. As regular resources, sheep and Galliformes represent 6% and 11%, respectively. As a supplementary resource, wild boar (adult and juvenile) were found in six scats, representing a total of 5% of F.O. Other supplementary resources were the horse (5% F.O.), the goat (5% F.O.), the pig (*Sus domestica*) (2% F.O.), the dog (2% F.O.) and the cat (2% F.O). Finally, as occasional resources were found the donkey (1% F.O.), the badger (*Meles meles*) (1% F.O.) and Small Mammals (1% F.O.). Lagomorphs were the only prey class that revelled statistically significant differences between the years covered by diet analysis in Sul do Douro study area (χ^2 = 7.35, 1 *g.l.*, $p < 0.05$).

3.2 Seasonal variation

Seasonal variation of wolf diet in Sul do Douro study area expressed in F.O. showed that Lagomorphs were the main food resource during all seasons, with values ranging from 46% of F.O. in Winter and 83% of F.O. in Summer (Figure 17; Appendix IX).

Although with less representativeness, the sheep was also found in all seasons, with the highest value corresponding to Winter (8%). The analysis of the effect of seasonality revealed statistically significant differences for the consumption Lagomorphs between Summer and Autumn (χ^2 = 5.85, 1 *g.l.*, p < 0.05) and between Winter and Summer (χ^2 = 6.42, 1 *g.l.*, p < 0.05). Besides Lagomorphs, Galliformes also present significant differences between Summer and Autumn (χ^2 = 5.01, 1 *g.l.*, p < 0.05).

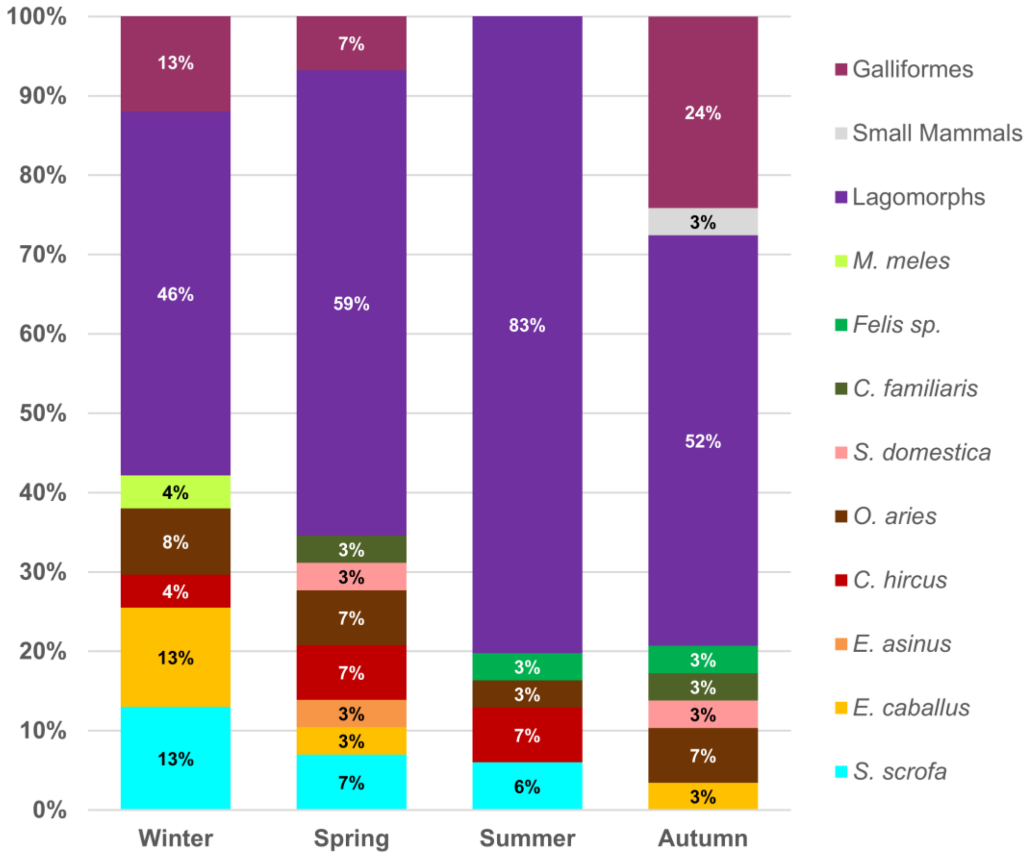


Figure 17 – Seasonal variation of wolf diet in Sul do Douro study area. Values expressed as F.O. of 91 scats collected between 2011 and 2013.

Regarding diet diversity and niche breath, Winter holds the highest values (H'=1.61; N.B.=0.46) and Summer the lowest (H'=0.69; N.B.=0.11) (Figure 18).

Niche overlap of trophic niche reveals similar exploitation between all seasons, considering that the values are close to 1. The more distinct exploitation was found between Autumn and Summer, presenting the lowest value (N.O.=0.894) (Appendix X).

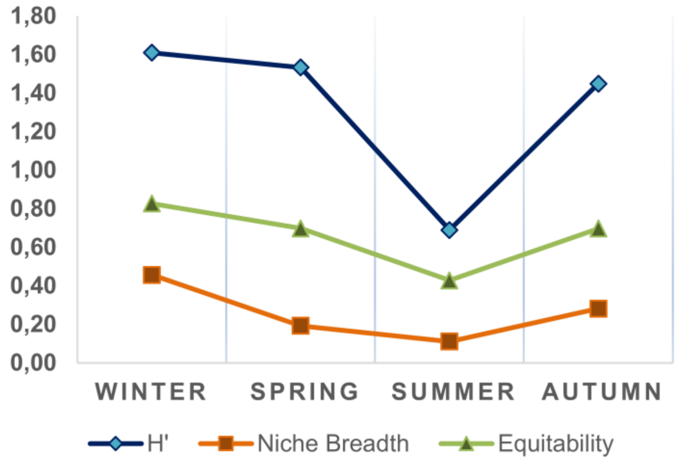


Figure 18 – Seasonal analysis of diet diversity (H' and Equitability – Shannon-Wever Index) and Niche Breadth (modified Simpson Index) for wolf diet in Sul do Douro study area.

3.3 Prey selection and feeding strategy

Prey selection measured by Ivlev's Index shows a strong preference towards lagomorphs and horses, and a little less towards goats, sheep, domestic pig and wild boar (Figure 19). For Galliformes, wolves showed a negative selection. Differences between the availability of prey and their consumption revealed highly significant differences horses, goats, sheep, domestic pig and wild boar (χ^2 test, p>0.05).

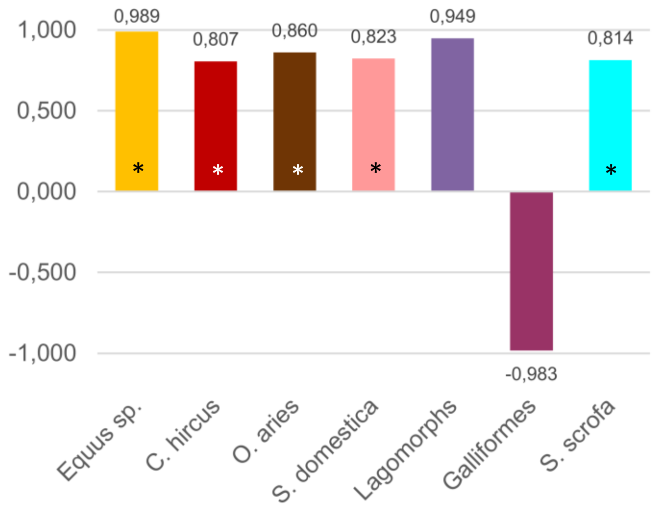


Figure 19 – Prey selection (Ivlev's Index) for wolf diet in Sul do Douro, according with the availability of main prey in the study area, and F.O. in wolf diet. The index varies between -1 (complete rejection of a species) and 1 (maximum positive selection). * Species with significant differences between occurrence in wolf diet and relative abundance.

The available data on wolf attacks to domestic ungulates declared to ICNF in Sul do Douro study area, during the same period covered by diet analysis (2011 to 2013), revealed a total of 305 casualties, being the horses the most affected prey, corresponding to 42% (N=127) of the attacks (Figure 20; Appendix XI). This values did not match completely with the results obtained in diet analysis, considering that the goat was the domestic ungulate more represented in wolf diet (N=7; 35%) but not in the declared attacks (N=61; 20%), as well as cattle, that was not found in wolf diet, but represents 15% (N=46) of livestock attacks.

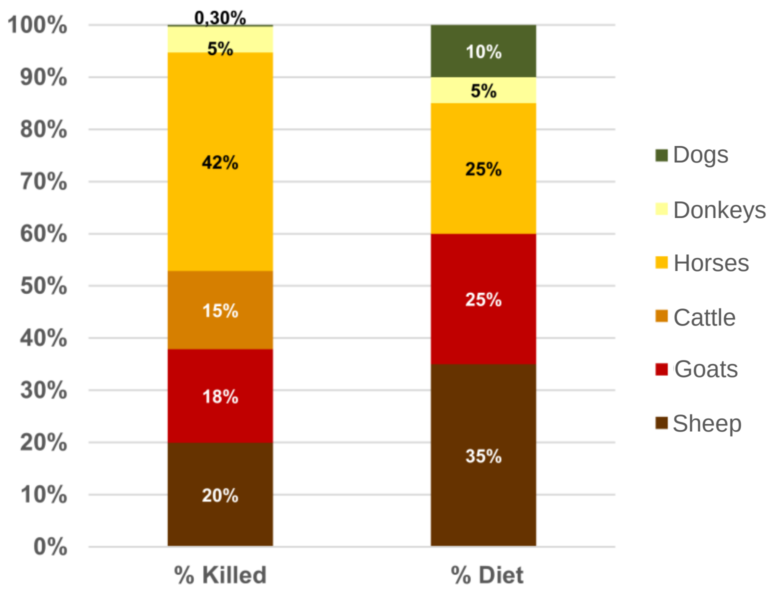


Figure 20 – Percentage of wolf attacks to each livestock species declared to ICNF in the parishes included in Sul do Douro study area (% Killed) in comparison with the percentage of prey detections in wolf scats (% Diet).

3.4 Temporal variation

Comparing the results of wolf diet obtained in this study with previous diet assessments in Sul do Douro study area, it becomes evident a decrease in domestic prey consumption, from being the main consumed prey (82% F.O.) to represent a mere 18% F.O. of the diet. (Figure 21). As for wild ungulates consumption, this prey class represents a constant low percentage along the years in this study area, with values ranging from 2% to 5%. The biggest change occurs in the presence of Lagomorphs in wolf diet. From 2002 to 2008, the F.O. of this prey class has been relatively constant, with an average of F.O. of 11%. The results obtained in this study, covering the period between 2011 and 2013, showed a high increase on their consumption thus becoming the main source of food (60% F.O.) The variation of carnivores and other prey items is relatively constant, maintaining similar percentages over the years (Figure 21).

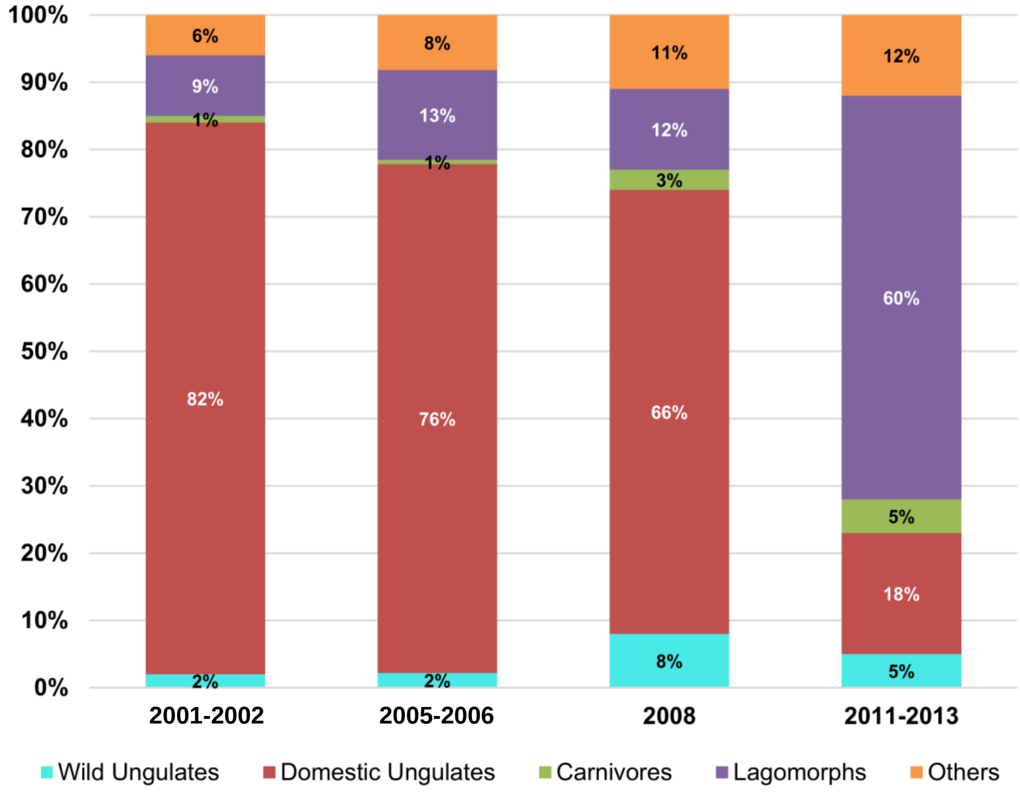


Figure 21 – Temporal variation of wolf diet along the years in Sul do Douro study area (Leomil pack). Values expressed in F.O. were obtain from: Quaresma (2002); Sobral (2006); Pinto (2008); This study (2011-2013). The category "Others" includes Small Mammals and Galliformes.

4. Peneda-Gerês – Soajo/Vez Packs

4.1 Feeding habits

In Peneda-Gerês (comprising Soajo and Vez packs), 118 scats genetically attributed to wolf were collected between 2008 and 2010. In the entire sample, the prey class of five scats (4%) were not possible to identify. For the remain 113 scats, prey classes identified in Peneda-Gerês study area included five ungulate species - two species of wild ungulates, represented by roe deer and wild boar, and three species of domestic ungulates represented by cattle, horses and goats (Table 6). The percentage of non-food items were not possible to obtain for the study area of Peneda-Gerês.

Within the five identified prey classes, domestic ungulates are the main category, representing the majority of the Biomass (96%), while wild ungulates represent only 4% of the consumed Biomass (Table 6). Horses (68% F.O.) stand out for being the only basic resource, followed by cattle (14% F.O.), and goat (7% F.O.) as regular resources. Other regular resource was roe deer, representing 7% of F.O. As a supplementary resource, the wild boar was found in only four scats, representing 4% of F.O. (Table 6).

Table 6 – Wolf diet in Peneda-Gerês study area (N=118 scats collected between 2008 and 2010), expressed in identified prey items, number of prey detections, Frequency of Occurrence, with correspondent classification of resource type, and Consumed Biomass.

	N	F.O. (%)	Resource	Biomass (%)
Wild Ungulates	**12**	**11**	**-**	**4**
C. capreolus	8	7	Regular	2
S. scrofa	4	4	Supplementary	2
Domestic Ungulates	**101**	**89**	**-**	**96**
B. taurus	16	14	Regular	21
E. caballus	77	68	Basic	73
C. hircus	8	7	Regular	2
Total	**113**	**100**	**-**	**100**

Horses were the only prey class that revelled highly statistical significant differences between the year 2008 and the year 2010 (χ^2 = 13.81, 1 *g.l.*, $p < 0.05$) and between the year 2009 and the year 2010 (χ^2 = 18.08, 1 *g.l.*, $p < 0.05$).

4.2 Seasonal variation

Seasonal variation of wolf diet in Peneda-Gerês study area revealed that Autumn is the season with a total consumption of domestic ungulates (100% F.O) Horses were the main food resource in all seasons, particularly Winter, comprising 82% of F.O. With less representativeness, cattle and goats were also found in all season, with the highest value corresponding to Summer for cattle (17% F.O.) and corresponding to Spring for goats (11% F.O.) Wild ungulates are represented in wolf diet mostly in Summer, with a total of 20% of F.O. (Figure 22 and Appendix XII). The analysis of the effect of seasonality revealed no significant differences (p>0.05 for all cases).

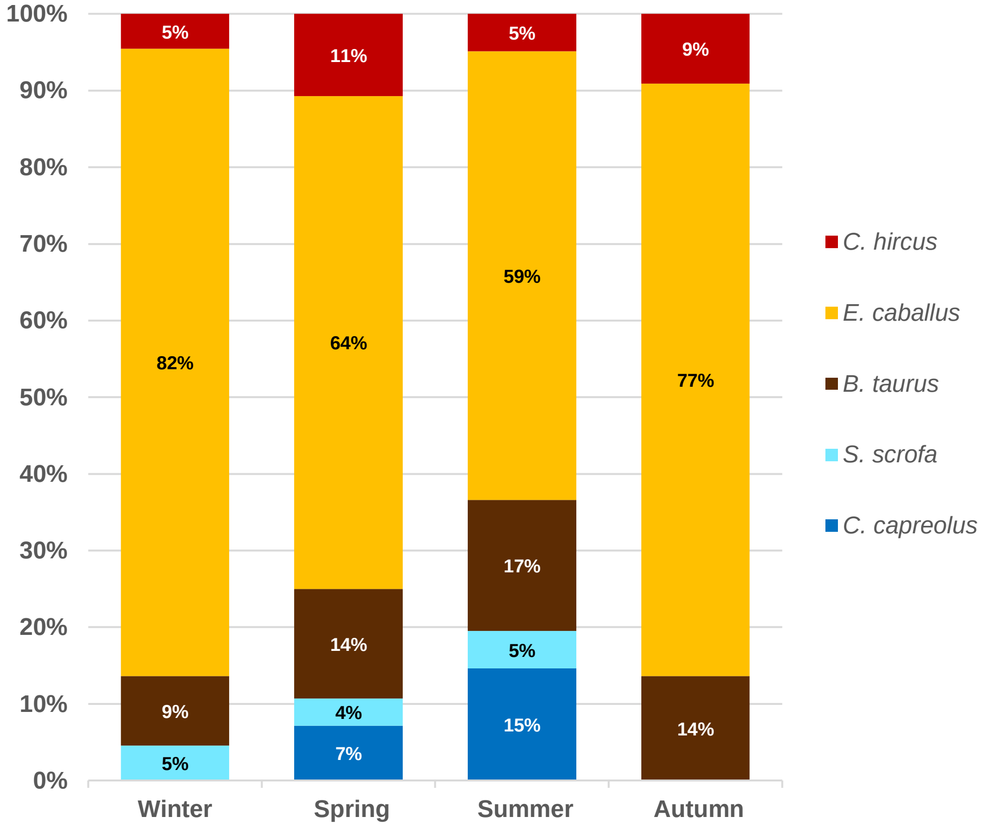


Figure 22 – Seasonal variation of wolf diet in Peneda-Gerês study area. Values expressed as F.O. of 118 scats collected between 2008 and 2010.

Diet diversity and niche breadth revealed that Summer was the season with the highest values (H'=1.19; N.B.=0.378), while Winter showed the lowest values (H'=0.66; N.B.=0.156) (Figure 23). However, no significant differences were detected in the χ^2 test for both indexes (χ^2 teste; p>0.05 for all values).

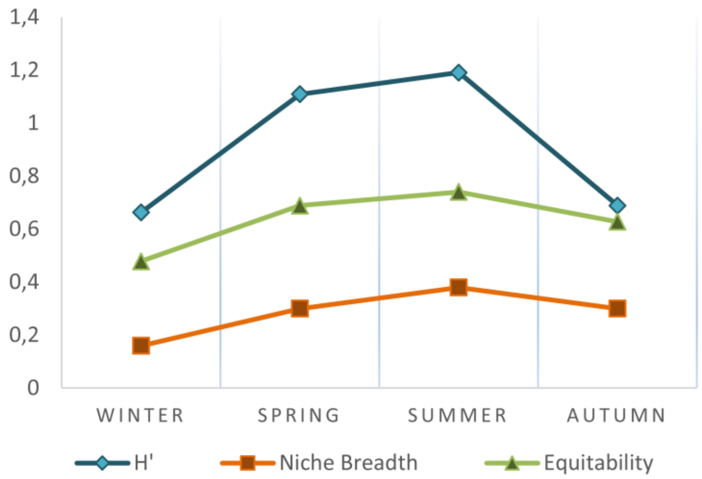


Figure 23 – Seasonal analysis of diet diversity (H' and Equitability – Shannon-Wever Index) and Niche Breadth (modified Simpson Index) for wolf diet in Peneda-Gerês study area.

Considering the overlap of trophic niche, all the values between seasons are very high, reflecting a very similar diet. The comparison between Spring and Autumn holds the highest value (N.O.=0.991), confirming the qualitative similarity of wolf diet in these two seasonal periods. The lowest value is found in the comparison between Winter and Summer (N.O.=0.958), suggesting a slightly dietary difference between both seasons (Appendix XIII).

4.3 Prey selection and feeding strategy

Prey selection measured by Ivlev's Index shows a clear and strong preference towards horses, and, despite the low F.O. in wolf diet, with less expression towards roe deer (Figure 24). For the other three ungulates, wolves showed a negative selection of cattle and goats, and an avoidance almost maximum towards wild boar. Differences between the availability of prey and their consumption revealed significant differences for goats and roe deer (χ^2 test, $p<0.05$).

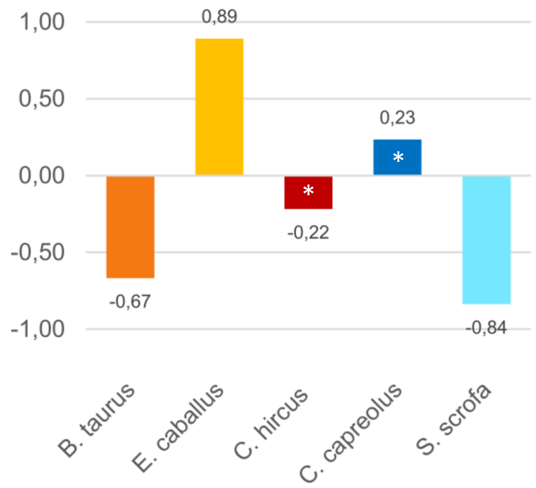


Figure 24 – Prey selection (Ivlev's Index) for wolf diet in Peneda-Gerês, according with the availability of main prey in the study area, and F.O. in wolf diet. The index varies between -1 (complete rejection of a species) and 1 (maximum positive selection). * Species with significant differences between occurrence in wolf diet and relative abundance.

The available data on wolf attacks to domestic animals in the parishes of the county of Arcos de Valdevez, belonging to the Peneda-Gerês study area, during the same period of time covered by diet analysis in this study, shows a total of 1474 casualties declared to ICNF that were attributed to wolf. Cattle was the most affected prey, representing almost half of the total kills (N=724; 49%) (Figure 25; Appendix XIV). This values did not match with the obtained results from diet analysis, considering that in wolf diet, the most represented prey was the horse (N=77; 76%), and there was no representation at all of sheep.

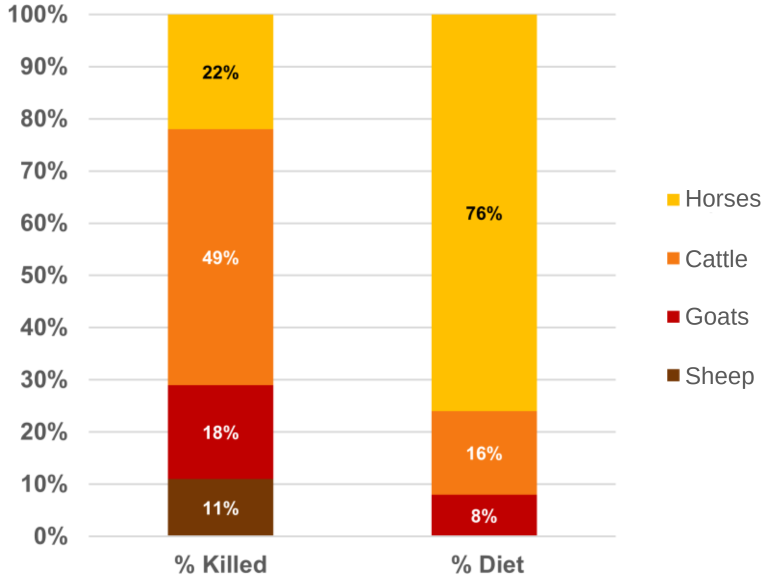


Figure 25 – Percentage of wolf attacks to each livestock species declared to ICNF in the parishes included in Peneda-Gerês study area (% Killed) in comparison with the percentage of prey detections in wolf scats (% Diet).

4.4 Temporal variation

Regarding the comparison between the results of wolf diet obtained in this study with previous diet assessments in Peneda-Gerês study area, between 1990 and 2004 it is observed a slight increase in the presence of wild ungulates in wolf diet, from 3% F.O. to 11% F.O (Figure 26). Between 2008 and 2010 the consumption of wild ungulates maintains constant in the 11% of F.O., but there is a slight increase on the consumption of domestic ungulates, from 85% of F.O. to 89%. Domestic ungulates, over two decades, although there are some variations, remain the main food resource and most consumed prey, with an average of 87% of F.O. This is the study area that suffers less variations of prey consumption, maintaining similar percentages for each prey item along the years. Carnivores, lagomorphs and other prey items, similar to wild ungulates, represent very small portions on wolf diet, maintaining that statute identical over the years (Figure 26).

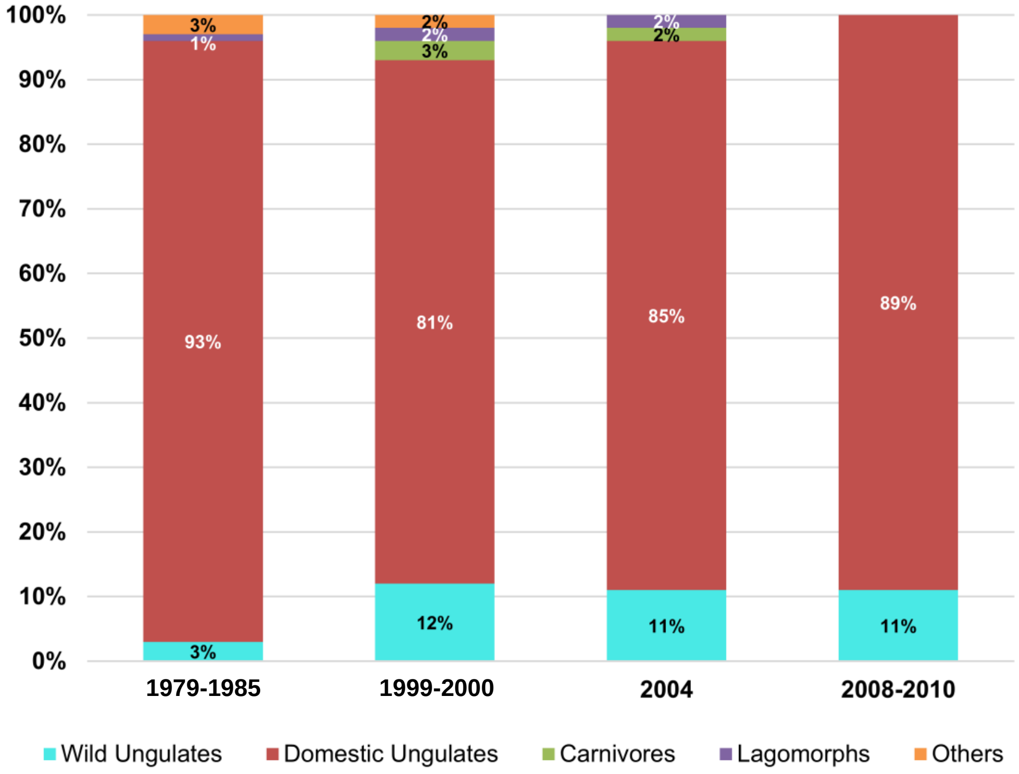


Figure 26 – Temporal variation of wolf diet along the years in Peneda-Gerês study area (Vez/Soajo packs). Values expressed in F.O. were obtain from: Petrucci-Fonseca (1990); Ferrão da Costa (2000); Guerra (2004); This study (2008-2010). The category “Others” includes Small Mammals and Galliformes.

II. Individual Patterns

1. Intra-pack individual variation

Intra-pack individual variation on wolf diet was assessed in 14 scats of four different wolves identified by non-invasive genetics (LAM 139, LAM 142, LAM 143 and LAM 144) and belonging to the same pack – Soajo pack – in Peneda-Gerês study area. Individuals are represented with a minimum of three recaptures in scats, collected in the same day. Results expressed in F.O. show evident differences between the prey species detected in each individual, although domestic ungulates represent most of the diet in all pack members (Figure 27; Appendix XV). In general, the most consumed prey was horse, representing approximately half of the diet for each wolf, except one (LAM 142) where this prey item was absent. Three distinct wolves had single occurrences of different species of wild prey, namely roe deer in LAM142, wild boar in LAM143 and carnivores (i.e. cat) in LAM139, always represented with smaller F.O. than domestic ungulates.

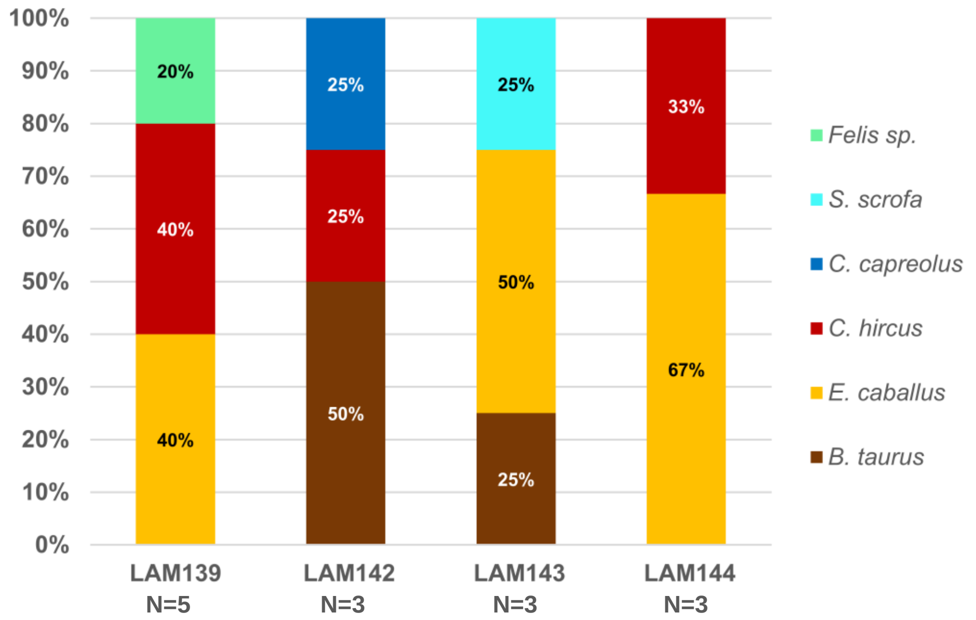


Figure 27 – Intra-pack individual variation on the diet of four different individual wolves identified by non-invasive genetics and belonging to Soajo pack (Peneda-Gerês). All scats (N=14) were collected in the same day. Values expressed in F.O. (N – number of samples analysed per individual).

2. INDIVIDUAL VARIATION ALONG TIME

Individual variation of diet along time was assessed in scats belonging to two distinct wolves (LSD 07 and LSD 53) from Leomil pack in Sul do Douro study area, and collected during a long temporal period. In particular, for wolf LSD 07 sample consists in 15 scats covering a period of 8 years (from 2008 to 2015) and for wolf LSD 53 sample consists in 16 scats covering a period of 5 years (from 2011 to 2015). Results expressed in F.O. show evident differences on the proportion and type of consumed prey items between years in wolf LSD 07 (Figure 28; Appendix XVI) while in wolf LSD 53, prey items were more similarly exploited along years (Figure 29; Appendix IX). However, in the years when sampling was coincident there is some similarity on consumed prey items between both wolves belonging to Leomil pack (Figure 28 and 29).

For wolf LSD 07, lagomorphs were in general the most consumed prey represented in four years normally with high values of F.O. This wolf also consumed livestock species (goats and sheep) as well as wild prey (wild boar and badger) with a variable occurrence across years (Figure 28; Appendix XVII).

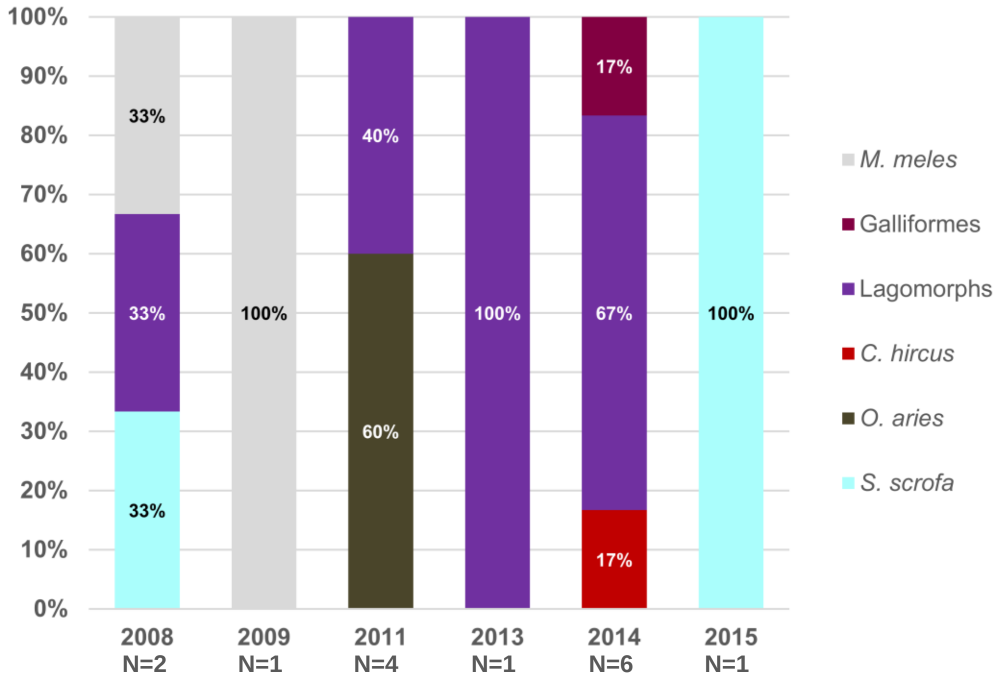


Figure 28 – Individual variation of diet along time in one single individual wolf (LSD 07), identified by non-invasive genetics, between 2008 and 2015 and belonging to Leomil pack (Sul do Douro). Values are expressed in F.O. (N – number of samples analysed per year).

Regarding wolf LSD 53, lagomorphs were also the most consumed prey, represented in all years included in this analysis and always with values of F.O. higher than 50%. Galliformes were the second most consumed prey while wild boar, horse and domestic pig represented a much smaller part of diet along the years, each prey species found in only one scat. (Figure 29; Appendix IX).

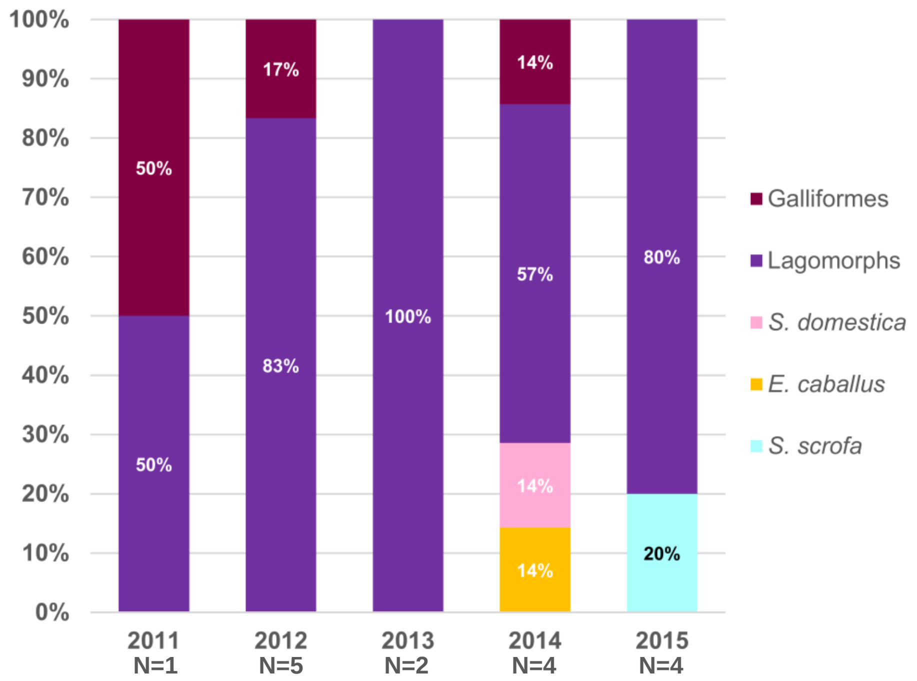


Figure 29 – Individual variation of diet along time in one single individual wolf (LSD 53), identified by non-invasive genetics, between 2011 and 2015 and belonging to Leomil pack (Sul do Douro). Values are expressed in F.O. (N – number of samples analysed per year).

CHAPTER V

DISCUSSION AND FINAL REMARKS

This study provided valuable insights on wolf trophic ecology in a multi-prey system dominated by domestic species, by assessing regional, temporal and individual patterns of variation in relation to livestock husbandry practices and wild prey availability. Besides focusing a topic with management relevance and worldwide implications, this study also provides updated information on wolf diet in Portugal. In fact, by focusing Montesinho, Sul do Douro and Peneda-Gerês areas, this study addresses the most current possible situation of wolf diet in the main wolf populations in Portugal and provides innovative insights on prey selection, feeding strategies (e.g. predation vs scavenging) towards domestic animals under different husbandry practices and the temporal evolution in the consumption of both wild prey and livestock.

I. INFLUENCE OF LIVESTOCK HUSBANDRY PRACTICES

Livestock predation by wolves is a worldwide concern and several studies, including this one, confirm that domestic ungulates can be a primary wolf prey, mainly depending on the type of husbandry practices (Barja, 2009; Iliopoulos *et al*., 2009; Vos, 2000; Torres *et al.*, 2015a). However, most studies across the entire wolf range document wolf predation on a few number of livestock species in extensive husbandry, mainly goats, sheep and cattle (Cuesta *et al*., 1991; Lançós, 1999; Vos, 2000; Torres *et al*., 2015a). In contrary, this study presents a much wider range of domestic animals consumed by wolves in each study area, including other livestock species under extensive grazing, such as horses, as well as domestic animals usually confined in intensive production farms (such as rabbits, poultry and domestic pigs).

The livestock husbandry practices in Peneda-Gerês study area are dominated by extensive grazing in mountain pastures, either with poor vigilance where rarely are used efficient livestock guarding dogs such as in goats and sheep, or in a free-ranging system with any vigilance, such as in horses and cattle (Vos, 2000; Pimenta *et al*., 2017). This system is characterized by multi-owner herds dominated by autochthonous breeds well adapted to local ecological conditions, that roam free in large numbers (Dondina *et al.*, 2015; Pimenta, 2017). Far from shelter and seldom confined with fences or in barns, without any shepherd or dog protection all year round, horses and cattle represent a particularly vulnerable, predictable and easy prey for wolves, that may lack most anti-predator tactics (Pimenta *et al*., 2017). As a consequence, Peneda-

Gerês is the area with higher number of damages on livestock in Portugal, which was confirmed by the evaluation of a predatory feeding strategy in this area based on declared losses to ICNF and the detected prey in scat analysis. Although those values may not match completely, domestic ungulates are presented as the main food resource in this study area, comprising almost 90% of wolf diet in Peneda-Gerês, which goes in agreement with the amount of declared kills of livestock. Even though the density of wild prey has been increasing over the last few decades in this region (Salazar, 2009; Álvares *et al.*, 2015, Torres *et al*., 2015b), wolves tend to maintain the preference over livestock, being the horse the most consumed prey and to which wolves should sustain a strong positive selection. Domestic animals are presented as an easy prey which consumption has increased almost 5% since the last study carried out in this area in 2004 (Guerra, 2004). Domestic ungulate, representing 85% F.O. of wolf diet, came to represent 89% F.O. of the current wolf diet. Diversity and density of wild prey is still not enough. Their low population size (1,57 ind/100 ha for roe deer; 10 ind./100 ha for wild boar; no available data on the population size of red deer in this area) and diversity turns livestock into the only abundant prey for wolves. The free-ranging system exercised in Peneda-Gerês is one of the factors that may explain the relative high percentage of attacks during Autumn and Winter (Pimenta *et al*., 2017), coincidently with the highest percentage on diet obtained in this study, especially for horses. During cold months, livestock tend to move to lower altitudes, but do not refugee in the village. With the concentration of livestock in a smaller range, wolf predation on those domestic animals is higher. During Spring and Summer there is a higher diversity in diet, that may be considered an opportunist behaviour from wolves, since it coincides with the birthing season of wild prey, and the individuals of this age are easier to capture (Salvador and Abad, 1987; Barja, 2009). Therefore, these are the seasons where the consumption of wild ungulates is more pronounced. Overall, in Peneda-Gerês, the consumption of wild prey represents very low percentages in wolf diet, ranging from 5% to 20%. It can be justified by the fact that this well adapted and opportunistic carnivore is prepared to kill overabundant and vulnerable prey, such as cattle and horses in Peneda-Gerês, having done so for centuries in these humanized environments (Iliopoulos *et al.*, 2009; Álvares *et al.,* 2014). Moreover, extensive husbandry systems in mountain pastures provides easier encounters between wolves and livestock, facilitating, and partially explaining, wolves' choice in hunting domestic ungulates. This constant abundance and easy access to domestic prey may justify the absence of temporal variations in their consumption.

In contrast with Peneda-Gerês, livestock husbandry practices in Montesinho study area consist in extensive grazing of sheep and goats with efficient vigilance (frequently with several large-sized livestock guarding dogs) and cattle under a semi-confined system, normally in individual-owned herds (Álvares, 2015; Pimenta *et al*., 2017). The domestic animals graze generally on private pastures close to shelters during the day, and are confined with fences or in barns during the night. This husbandry system appears to suffer much lower losses to wolf predation (Pimenta *et al*., 2017), fact that is well reflected in the results from scat analysis, where livestock comprises only 6% of wolf diet in Montesinho study area. Montesinho, together with being the region with higher density of wild prey, is the area with less damages to livestock (Álvares *et al*., 2015). The only detected domestic prey in scat analyses from Montesinho, were goats, while only sheep were represented in declared attacks attributed to wolves. This discrepancy may happen for several reasons. Primarily, this analysis does not include information on livestock attacks in the neighbouring areas located in Spain, which comprises a reasonable portion of the home ranges of sampled packs in Montesinho study area. Furthermore, the absence of sheep remains in wolf diet may be due to low coverage of the scat sampling of this study in order to detect an occasional food resource or, in other hand, because owners are obliged to cover or remove the dead prey in order to facilitate evaluation by authorities for damage compensation and for sanitary reasons, a practice that often prevents wolves from feeding on killed livestock. Another cause could be that shepherds, in order to receive the compensation amount for their losses on livestock, occasionally attribute wrongly the cause of death to wolves (Vos, 2000; Llaneza and López-Bao, 2015). On the other hand, the absence of goats – a detected prey item – in the declared attacks to livestock may also be justified by the fact that some losses in livestock are not reclaimed by their owners. It may also be result of scavenging behaviour by wolves on goat carcasses killed by other causes and illegally dumped far from villages. Moreover, the higher percentage of goats detected in wolf scats in Montesinho study area was in Summer, when these animals cover wider areas under extensive grazing in mountainous and more inaccessible regions, which may prevent owners of finding remains of goat carcasses killed by wolves and show them to the authorities for the right of compensation (Vos, 2000).

In Sul do Douro, is practiced an extensive husbandry for sheep and goats, a semi-confined system for cattle, horses and donkeys and an intensive production in closed farms for domestic pig, rabbits and poultry (Torres *et al.,* 2015a; Pimenta *et al.,* 2017). This variety in husbandry practices involves different levels of prey availability and exposure to wolf predation. However most of these domestic animals were detected in wolf diet suggesting distinct feeding strategies based on either a predatory behaviour for ungulates or a scavenging behaviour for the remain domestic species. Although the numbers of declared losses to ICNF are not so high as Peneda-Gerês, this study area is also a relevant part in livestock damages attributed to wolves (Vos, 2000; Álvares, 2015; Torres *et* al., 2015a; Pimenta *et al*., 2017). Although not totally coincident, the declared casualties and detected prey in wolf scats from this study area demonstrated that domestic pigs, poultry and, particularly, domestic rabbits may be consumed on dumpsites with carcasses from intensive farms. The consumption of lagomorphs in the study area of Sul do Douro appears to be essential to the survival of wolves at the present time. In previous studies (Quaresma, 2002; Sobral, 2006; Pinto, 2008), this resource was not so representative in wolf diet (maximum value of 13% F.O.) as seems to be now, considering it represents 60% of F.O. The availability of this food resource depends on the accessibility to wolves of intensive farms and respective dump sites where carcasses are left (Alexandre *et al.,* 2000). Summer presents the lowest values of domestic prey in the detected prey in wolf scats (10% F.O). On the other hand, this is the season with the highest consumption of lagomorphs, which may be related with changes in the activity of wolves near farms, where they may search more often for food, by scavenging in animals remains left in dumps (Llaneza and López-Bao, 2015). It may also be correlated with a seasonal change of the territory used by the wolf pack, coincident with the period when wolf pups require larger food intake (Urios *et al*., 2000). It is remarkable the difference along the years on the consumption pattern on domestic ungulates (decreasing) and lagomorphs (increasing) in wolf diet from Sul do Douro. Changes in husbandry practices, implemented measures to prevent wolf attacks and the exodus of the rural population may be correlated with those differences (Vos, 2000; Torres *et al.,* 2015a). Domestic ungulates in extensive grazing systems are decreasing (Vos, 2000), which may represent a big threat to wolves, considering that currently their food income and survival relies mostly on human activities and such as carcasses on dumpsites. As an alternative, dog and cats are occasionally part of wolf diet as a supplementary resource. That fact was confirmed by the declared loss to ICNF of a dog, prey class also found in the analysis of wolf scats. This may be justified by the highly anthropogenic changes of this region, which bring wolves to the proximity of humanized areas. However, with no availability of wild prey, this can ultimately lead to low reproduction rates on wolves, meaning their eventual disappearance from this region, which can ultimately result in local extinctions (Alexandre *et al*., 2000; Vos, 2000; Torres *et al.,* 2015a).

II. INFLUENCE OF WILD PREY AVAILABILITY

Wild prey are expected to be the primary prey of wolves and helpful in preventing livestock depredation, when existing in high abundance and diversity (Smietana and Klimek, 1993; Meriggi and Lovari, 1996; Poulle *et al.,* 1997; Boitani, 2000; Barja, 2009;

Meriggi *et al.*, 2011). This is clearly confirmed in Montesinho study area, where a diverse community of wild ungulates (red deer, roe deer and wild boar) are wolves main prey, comprising currently more than 90% of consumed Biomass and with an increasing trend during the last few decades. To achieve this ecological scenario unique within Portuguese wolf range, is necessary a combination of high abundance and diversity of wild prey together with efficient vigilance of livestock, which moulds the preference of wolves for its natural prey. In Montesinho, the red deer is the most important wild prey species. Although it is not the most abundant one (Appendix I), according with the results obtained from the analysis of wolf scats, there is a clear positive selection towards this species, which consumption has been increasing since the last studies conducted in this area, from representing a mere 4% of F.O in wolf diet (Moreira, 1992) to became the main consumed prey (40% F.O). The second is the wild boar. The preference over this prey can be related with the fact that this species lives in large groups, being thus easily detected (Meriggi *et al*., 2011). However, a single wolf, or just few wolves, are unlikely to be able to handle a healthy adult wild boar (Barja, 2009), which may justify the fact why this species is not the main wolf prey in any of the study areas, even being the wild ungulate that exists in higher densities (Appendix I). The third species in terms of importance in Montesinho is the roe deer, which is very widespread and occurring in high densities. Given that the size of wolf prey is related to the size of packs (Thurber and Peterson, 1993; Schmidt and Mech, 1997; Hayes *et al*., 2000; Jedrzejewski *et al*., 2002), roe deer can easily satisfy the food requirements of a small pack (Jedrzejewski *et al*., 2002; Meriggi *et al*., 2011), and become a regular or supplementary wild prey, as it is the case in Montesinho and Peneda-Gerês, respectively. However, the roe deer, even existing at reasonable densities, can be more difficult to detect, as it is a solitary and territorial species (Meriggi *et al*., 1996), justifying the negative selection obtained in the results for Montesinho.
The results confirmed that natural prey is a major missing element in wolf´s ecology in Peneda-Gerês, and even more in Sul do Douro, considering that in many regions, the restoration of wild ungulates led to a significant decrease in livestock damages, (Meriggi and Lovari, 1996; Poulle *et al.,* 1997; Breitenmoser, 1998; Gazzola *et al.,* 2005; Zlatanova *et al.*, 2014).

III. MANAGEMENT RECOMMENDATIONS AT REGIONAL SCALE

Understanding what may influence the feeding habits and predatory impacts of wolves is of great importance in outlining effective strategies for their management and conservation. Therefore, the knowledge of wolf's trophic ecology is essential, in order to implement correct and adapted measures.

The big issue about wolf conservation in Portugal focus on the problematic around the conflict with humans, due to the damages caused in domestic animals (Vos, 2000; Pimenta *et al*., 2017). Understanding what drives the wolf's prey preferences in areas where different potential prey species coexist, may be extremely useful in reducing the number of attacks in livestock (Barja, 2009). Other studies focused on wolves and other large carnivores have demonstrated that predation on domestic animals may remain frequent in areas with high densities of wild prey, if livestock is locally abundant and protective methods are not effectively enforced and respected (Poulle *et al.,* 1997; Llaneza *et al.*, 2000). Furthermore, if adequate husbandry techniques are applied, livestock predation may be rare despite low densities of wild prey (Soh *et al*., 2014). It is urgent to implement better prevention measures, adapting the strategies to each area, taking into account certain aspects, such as the type of husbandry system.

Surveillance of livestock by shepherds is probably the most effective way of protecting livestock from wolf attacks (Iliopoulos *et al.*, 2009), combining with the use of predator-proof enclosures, especially during night time, and livestock guarding dogs. The size of the herds is also something to have in consideration (Vos, 2000). Larger herds are more difficult to control and easily scattered in larger areas, providing the increase of encounters with wolves and thus the possibility of being predated (Mech *et al.,* 2000; Bradley and Pletscher, 2005; Dondina *et al.*, 2014). It is also frequent that these larger herds are loosely attended by their owners, increasing the vulnerability to animals straying from their herds or becoming in poor health conditions and, ultimately, predated by wolves (Pimenta *et al.,* 2017). Peneda-Gerês represents the main priority for the implementation of efficient damage protection measures, considering the free-ranging husbandry system applied in this area, where cattle and horses roam freely and unprotected during all year (Vos, 2000; Álvares *et al*., 2015; Pimenta *et al*., 2017). Is also necessary to have in consideration that in certain seasons of the year the protection measures should be reinforced, particularly during Spring and early Summer, in the wolves' post-weaning period and livestock newborn births, when the rate and severity of wolf attacks are higher (Bradley and Pletscher, 2005; Iliopoulos *et al*., 2009; Dondina *et al.,*2014).

As stated before, in Portugal, wolves natural prey are a missing element in wolf habitat. In this context, promoting the natural expansion and, whenever necessary, the reinforcement of reintroduction programs of wild prey, focusing in red deer and roe deer, should be a priority. In a medium to long-term, it will allow the wolf to regain the choice for its natural prey, which may be particularly relevant in areas where changing the husbandry system involve a significant effort by the rural population (Boitani, 1992; Pimenta *et al.,* 2017). At this moment, the survival of wolf population in Sul do Douro region is mainly dependent of intensive farms and access to carrion, especially of domestic rabbits. The closure of those farms, combined with the scarcity of wild prey, can ultimately result in wolves' low productivity and local extinctions (Alexandre *et al.,* 2000). More recently, feral and stray dogs came to aggravate the situation. Therefore, the management plans should take into account the control of feral dogs, that cause livestock damages, wrongly attributed to wolves, and also constitute a source of food competition for this top predator.

Finally, the entire compensation programmes for livestock losses should be reviewed. They should start to have as strict requisite the use of damage preventive measures adopted by livestock owners, instead of simply compensate the losses. This may help to reduce the enormous amount of money spent every year in compensations schemes. Since compensation programmes are not always the most effective measure to reduce predation and conflicts (Vos, 2000; Dyar and Wagner, 2003; Gazzola *et al.,* 2008), the investment in well trained guarding dogs, implemented in numbers adapted to flock size, may be a good alternative. They play a vital role in preventing livestock depredations, as wolves appear to avoid areas with guarding dogs (Smith *et al.,* 2000; Iliopoulos *et al*., 2009; Torres *et al*., 2015a).

The management projects for conservation of wolf in Portugal should be re-evaluated. An effective management of conflicts between wolf and husbandry systems constitutes a key element of a viable strategy for the conservation of this species. In this context, increase wild prey numbers, improve efficient livestock husbandry practices and eliminate monetary incentives that reward poor management practices are priorities (Boitani, 2000; Mech *et al*., 2000; Treves *et al.*, 2004; Bradley and Pletscher, 2005; Dondina *et al*., 2014).

IV. CONCLUSIONS AND FUTURE RESEARCH

This study provides relevant information on wolf trophic ecology in a multi-prey system regarding spatial, temporal and individual patterns of variation, using Portuguese wolves as a model. General results on wolf diet at regional level seem to be in agreement with previous studies (Petrucci-Fonseca, 1990; Moreira, 1992; Pimenta, 1998; Vos, 2000; Quaresma, 2002; Pinto, 2008; Álvares, 2011; Torres *et al.,* 2015a). However, the present study adds updated information on the current diet of wolves from three population nuclei from Portugal as well as provides innovative information on prey selection, feeding strategies and temporal trends on wolf feeding ecology during the last few decades. Furthermore, this study involves the first assessment of wolf diet in Portugal based in the analysis of scats genetically validated, which brings more confidence to the results, when comparing with the previous studies of wolf diet, all based on scats without genetic validation.

Regarding the individual patterns of variation, although being a preliminary approach considering the small sample size and the lack of knowledge on the social status of the sampled wolves, it provides valuable and innovating insights. In fact, the differences found in the diet of individuals belonging to the same pack, suggests individual differences in foraging behaviours probably due to individual preferences or low group cohesion during search for food (Schmidt and Mech, 1997; Mech, 1999). As for the individual variation along time in Sul do Douro, results also suggest different foraging behaviours and a certain preference towards lagomorphs, the most consumed prey for both wolves. Moreover, the fact that the wolf LSD 07 fed manly on livestock from extensive grazing while the wolf LSD 53 fed mainly on animals from intensive grazing may reflect different feeding strategies, such as predation for the wolf LSD 07 and scavenging behaviour for the wolf LSD 53 (Llaneza and López-Bao, 2015). Once again, even being a preliminary approach, individual variation on wolf diet appears to be a subject that has an enormous potential as a research avenue, considering it is an unexplored field of carnivore ecology worldwide that can bring important implications regarding the management of livestock depredation.

In parallel with studies of wolf trophic ecology, a regular monitoring of wild prey populations should be taken in consideration. If one of the main conservation actions to recover wolf populations and minimize livestock damages should focus on the reintroduction of wild prey to their former habitat, it is important to be aware of their current status, such as distribution, abundance and habitat selection.

The study of wolf trophic ecology through the analysis of scats, although it is the most used technique (Ciucci *et al.,* 1996; Klare *et al.*, 2011), it may not be the most effective, adequate and complete one, considering it does not allow to determine with certainty some aspects, such as feeding strategy (predation *vs* scavenging) and prey age (Cuesta *et al*., 1991). Telemetry, through the use of localization clusters, was the ideal methodology for future projects focusing wolf predation. That method would enable better knowledge regarding wolf feeding behaviour and would also be an essential tool for the study of individual patterns. Moreover, with the possibility of going to the known killing site, this would make it possible to determine the age of the prey by direct observation (Barja, 2009).

Wolf population in Portugal is dependent of essential factors for their survival in humanized and impoverished areas, such as reinforcement of wild ungulates, promoting preventive measures and surveillance of livestock. Those actions should have as long-term goal driving wolves to become more dependent on the consumption of wild prey, transmitting, in turn, that behaviour to their offspring (Barja, 2009). As a consequence, livestock depredation, hence the conflict with humans, would become a minor problem, guaranteeing the long-term conservation of wolves in human-dominated landscapes, such as Portugal.

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APPENDIX

Appendix I - Estimated population size of the main species of domestic and wild ungulates represented in wolf diet for each study area and declared losses of livestock to ICNF due to wolf attack (based in information from official statistics and published data at the level of parishes included in each study area: see methods section for further details).

Study Area	Prey species	Year	Population size	Source
Montesinho 148.33 km^2	*C. hircus*	2009	127	INE, 2011
	Lagomorphs	2009	341	INE, 2011
	C. elaphus	2010-2013	460	Santos, 2015
	C. capreolus	2012/2013	722	Valente *et al*, 2014
	S. scrofa	Current date	1483	Terras de Sicó, 2017; CONFAGRI, 2009
Sul do Douro 229.28 km^2	*E. caballus*	2009	122	INE, 2011
	C. hircus	2009	1978	INE, 2011
	O. aries	2009	1983	INE, 2011
	S. domestica	2009	698	INE, 2011
	Lagomorphs	2009	16439	INE, 2011
	S. scrofa	Current date	2293	Terras de Sicó, 2017; CONFAGRI, 2009
Peneda-Gerês 304.14 km^2	*E. caballus*	2009	1144	INE, 2011
	B. taurus	2009	4727	INE, 2011
	C. hircus	2009	1120	INE, 2011
	C. capreolus	2003	478	Ferreira, 2003
	S. scrofa	Current date	3041	Terras de Sicó, 2017; CONFAGRI, 2009

Study Area	Prey class	Declared losses	Study Area	Prey class	Declared losses	Study Area	Prey class	Declared losses
Montesinho 2016-2017	Sheep	2	Sul do Douro 2011-2013	Sheep	61	Peneda-Gerês 2008-2010	Sheep	158
	Goats	0		Goats	56		Goats	270
	Cattle	0		Cattle	46		Cattle	724
	Horses	0		Horses	127		Horses	322
	Donkeys	0		Donkeys	14		Donkeys	0
	Dogs	0		Dogs	1		Dogs	0

Appendix II – Prey items identified in the trial performed to assess dietary differences between analysing a small portion of a scat (≈25%, as collected for genetic validation) vs. the main portion of the scat, using two different datasets collected in Montesinho (BR3 to BR 77) and in Alto Minho (GA1 to GA33) (NIP – No identified prey; Green shade – scats with detected differences in prey items).

ID	Identified Prey - Main Portion	Identified Prey - Small Portion (≈25%)
BR3	*Cervus elaphus*	*Cervus elaphus*
BR4	*Capra hircus*	*Capra hircus*
BR8	*Cervus elaphus*	*Cervus elaphus*
BR10	*Capra hircus*	*Capra hircus*
BR13	*Sus scrofa*	*Sus scrofa*
BR14	*Cervus elaphus*	*Cervus elaphus*
BR16	*Felis silvestris*	*Felis silvestris; Cervus elaphus*
BR17	*Capra hircus; Cervus elaphus*	*Capra hircus; Cervus elaphus*
BR18	*Cervus elaphus*	*Cervus elaphus*
BR19	*Cervus elaphus*	*Cervus elaphus*
BR20	*Felis silvestris; Cervus elaphus*	*Felis silvestris; Cervus elaphus*
BR26	*Cervus elaphus*	*Cervus elaphus*
BR27	*Cervus elaphus*	*Cervus elaphus*
BR28	*Cervus elaphus*	*Cervus elaphus*
BR29	*Felis silvestris*	*Felis silvestris; Cervus elaphus*
BR30	*Felis Silvestris*	*Felis silvestris*
BR31	*Cervus elaphus*	*Cervus elaphus*
BR32	*Cervus elaphus*	*Cervus elaphus*
BR33	*Capra hircus*	*Capra hircus*
BR34	*Cervus elaphus*	*Cervus elaphus*
BR37	*Felis silvestris*	*Felis silvestris*
BR38	*Felis silvestris*	*Felis silvestris*
BR39	*Cervus elaphus*	*Cervus elaphus*
BR41	*Cervus elaphus*	*Cervus elaphus*
BR42	*Sus scrofa*	*Sus scrofa*
BR52	*Cervus elaphus*	*Cervus elaphus*
BR61	*Felis silvestris; Cervus elaphus*	*Cervus elaphus; Rattus rattus*
BR62	*Sus scorfa; Cervus elaphus; Felis silvestris*	*Sus scrofa; Cervus elaphus; Felis silvestris*
BR65	*Sus scrofa; Cervus elaphus*	*Cervus elaphus; Sus scrofa*
BR66	*Capra hircus*	*Capra hircus*
BR71	*Cervus elaphus*	*Cervus elaphus*
BR72	*Sus scrofa*	*Sus scrofa*
BR77	*Cervus elaphus*	*Cervus elaphus*

ID	Identified Prey - Main Portion	Identified Prey - Small Portion (≈25%)
GA1	*Bos taurus*	*Bos taurus*
GA2	*Bos taurus*	*Bos taurus*
GA3	*Equus caballus*	*Equus caballus*
GA4	*Equus caballus*	*Equus caballus*
GA5	*Equus caballus; Capreolus capreolus; Rattus rattus;* NIP	*Equus caballus; Capreolus capreolus; Rattus rattus;* NIP
GA6	*Bos taurus*	*Bos taurus*
GA7	*Equus caballus*	*Equus caballus; Cervus elaphus*
GA8	*Bos taurus*	*Bos taurus*
GA9	NIP	NIP
GA10	*Bos taurus*	*Bos taurus*
GA11	*Equus caballus*	*Equus caballus*
GA12	*Capra pyrenaica*	*Capra pyrenaica*
GA13	*Equus caballus*	*Equus caballus*
GA14	*Bos taurus*	*Bos taurus*
GA15	NIP	NIP
GA16	*Equus caballus*	*Equus caballus*
GA17	*Equus caballus*	*Equus caballus*
GA18	*Equus caballus*	*Equus caballus*
GA19	*Bos taurus*	*Bos taurus*
GA20	*Equus caballus*	*Equus caballus*
GA21	*Canis familiaris*	*Canis familiaris*
GA22	*Bos taurus*	*Bos taurus*
GA23	*Equus caballus*	*Equus caballus*
GA24	*Capra hircus; Capreolus capreolus*	*Capra hircus; Capreolus capreolus*
GA25	*Felis silvestris*	*Felis silvestris*
GA26	*Equus caballus*	*Equus caballus*
GA27	*Equus caballus*	*Equus caballus*
GA28	*Equus caballus*	*Equus caballus*
GA29	*Equus caballus*	*Equus caballus*
GA30	*Equus caballus*	*Equus caballus*
GA31	*Equus caballus*	*Equus caballus*
GA32	*Equus caballus*	*Equus caballus*
GA33	*Equus caballus*	*Equus caballus*

Appendix III – Number of occurrences and percentage of the non-food material items, identified macroscopically, for Montesinho and Sul do Douro. These items consist in wolf hairs, non-identified material, bones, purgative plants, mineral materials, plant leaves, insects and garbage. The number of occurrences and percentages of these items were not possible to obtain for Peneda-Gerês study area.

Non-food material	Wolf hairs		Non-identified material		Bone Material		Purgative plants	
	N	%	N	%	N	%	N	%
Montesinho	20	20%	0	0%	69	70%	5	5%
Sul do Douro	23	25%	1	1%	75	82%	1	1%
Peneda-Gerês	The number of occurrences and percentages of non-food items were not possible to obtain for this study area							

Non-food material	Mineral materials		Plant leaves		Insects		Garbage	
	N	%	N	%	N	%	N	%
Montesinho	80	82%	85	87%	1	1%	2	2%
Sul do Douro	88	97%	81	89%	2	2%	2	2%
Peneda-Gerês	The number of occurrences and percentages of non-food items were not possible to obtain for this study area							

Appendix IV – Average weights of each prey class detected in wolf diet and used to estimate Consumed Biomass. The weight of the prey class *Felis* sp. is a mean value between the weights of the wild cat (*Felis silvestris*) and the domestic cat (*Felis catus*).

Prey Class			Mean Weight (Kg)	References
Wild Ungulates	*Cervus elaphus*		97,5	Carranza (2011)
	Capreolus capreolus		24,0	Pimenta (1998)
	Sus scrofa	Juvenil	22,0	Llaneza *et al* (1996)
		Adult	67,0	
	Capra pyrenaica		60,5	Cassinelo and Acevedo (2011)
Domestic Ungulates	*Equus caballus*		200,0	Álvares (1995)
	Equus asinus		180,0	Álvares (1995)
	Bos taurus		300,0	ICN, 1997
	Sus domestica		135,0	Bastos (2001)
	Capra hircus	Montesinho	28,0	Moreira (1992)
		Sul do Douro and Peneda-Gerês	25,0	DRAEDM (1993)
	Ovis aries	Montesinho	28,0	Moreira (1992)
		Sul do Douro and Peneda-Gerês	20,0	DRAEDM (1993)
Carnivores	*Canis familiaris*		15,0	Moreira (1992)
	Felis sp.		3.9	Moreira (1992); López-Martín *et al* (2011)
	Meles meles		7,3	Revilla *et al* (2011)
Lagomorphs			1,5	Moreira (1992)
Small Mammals			0,02	Macdonald and Barret (1993)
Galliformes			1,85	Quaresma (2002)

Appendix V – Overlap of trophic niche (Pianka's Index) between wolf diet in three study areas: Montesinho, Sul Do Douro and Peneda-Gerês.

	Montesinho	Sul do Douro	Peneda-Gerês
Montesinho	-		
Sul do Douro	0,086	-	
Peneda-Gerês	0,066	0,082	-

Appendix VI - Seasonal variation of wolf diet in Montesinho study area (Minas/Rachas packs), expressed in Frequency of Occurrence (F.O) (n – number of prey detections in 98 scats collected during 2016/2017; N - number of scats analysed per season).

		Spring N=35		Summer N=24		Autumn N=13		Winter N=26	
Prey Class		**n**	**F.O. (%)**	**n**	**F.O. (%)**	**n**	**F.O. (%)**	**n**	**F.O. (%)**
Wild Ungulates		**36**	**95%**	**16**	**62%**	**15**	**83%**	**41**	**93%**
C. elaphus		16	42%	15	58%	7	39%	13	30%
C. capreolus		9	24%	0	0%	2	11%	7	16%
S. scrofa	Juvenil	3	8%	0	0%	0	0%	7	16%
	Adult	8	21%	1	4%	6	33%	14	32%
Domestic Ungulates		**1**	**3%**	**4**	**15%**	**1**	**6%**	**1**	**2%**
C. hircus		1	3%	4	15%	1	6%	1	2%
Carnivores		**0**	**0%**	**6**	**23%**	**2**	**11%**	**1**	**2%**
Felis sp.		0	0%	6	23%	2	11%	1	2%
Lagomorphs		**1**	**3%**	**0**	**0%**	**0**	**0%**	**1**	**2%**
Total		38	100%	26	100%	18	100%	44	100%

Appendix VII - Overlap of trophic niche (Pianka's Index) of wolf diet in Montesinho study area.

	Winter	**Spring**	**Summer**	**Autumn**
Winter	-			
Spring	0,913	-		
Summer	0,527	0,713	-	
Autumn	0,938	0,947	0,786	-

Appendix VIII - Number (N Killed) and percentage (%K) of wolf attacks to each livestock species declared to ICNF in the parishes included in Montesinho study area in comparison with the number of prey detections (N Diet) and respective proportion (% D) in wolf scats, during the time period covered by diet analysis.

	N Killed	**% K**	**N Diet**	**% D**
Sheep	2	100	0	0
Goats	0	0	7	100
Cattle	0	0	0	0
Horses	0	0	0	0
Donkeys	0	0	0	0
Dogs	0	0	0	0
Total	**2**	**100**	**7**	**100**

Appendix IX - Seasonal variation of wolf diet in Sul do Douro study area (Leomil pack), expressed in Frequency of Occurrence (F.O.) (n – number of prey detections in 91 scats collected during 2011/2013; N - number of scats analysed per season).

		Spring N=23		Summer N=26		Autumn N=22		Winter N=20	
Prey Class		**n**	**F.O. (%)**	**n**	**F.O. (%)**	**n**	**F.O. (%)**	**n**	**F.O. (%)**
Wild Ungulates		**2**	**7%**	**1**	**3%**	**0**	**0%**	**3**	**13%**
Sus scrofa	Juvenil	1	3%	0	0%	0	0%	1	4%
	Adult	1	3%	1	3%	0	0%	2	8%
Domestic Ungulates		**6**	**21%**	**3**	**10%**	**4**	**14%**	**6**	**25%**
Equus caballus		1	3%	0	0%	1	3%	3	13%
Equus asinus		1	3%	0	0%	0	0%	0	0%
Capra hircus		2	7%	2	7%	0	0%	1	4%
Ovis aries		2	7%	1	3%	2	7%	2	8%
Sus domestica		1	3%	0	0%	1	3%	0	0%
Carnivores		**1**	**3%**	**1**	**3%**	**2**	**7%**	**1**	**4%**
Canis familiaris		1	3%	0	0%	1	3%	0	0%
Felis sp.		0	0%	1	3%	1	3%	0	0%
Meles meles		0	0%	0	0%	0	0%	1	4%
Lagomorphs		**17**	**59%**	**24**	**83%**	**15**	**52%**	**11**	**46%**
Small Mammals		**0**	**0%**	**0**	**0%**	**1**	**3%**	**0**	**0%**
Galliforms		**2**	**7%**	**0**	**0%**	**7**	**24%**	**3**	**13%**
Total		29	100%	29	100%	29	100%	24	100%

Appendix X – Overlap of trophic niche (Pianka's Index) of wolf diet in Sul do Douro study area.

	Winter	**Spring**	**Summer**	**Autumn**
Winter	-			
Spring	0,954	-		
Summer	0,904	0,980	-	
Autumn	0,924	0,933	0,894	-

Appendix XI - Number (N Killed) and percentage (%K) of wolf attacks to each livestock species declared to ICNF in the parishes included in Sul do Douro study area in comparison with the number of prey detections (N Diet) and respective proportion (% D) in wolf scats, during the time period covered by diet analysis.

	N Killed	% K	N Diet	% D
Sheep	61	20	7	35
Goats	56	18	5	25
Cattle	46	15	0	0
Horses	127	42	5	25
Donkeys	14	5	1	5
Dogs	1	0,3	2	10
Total	**305**	**100**	**20**	**100**

Appendix XII - Seasonal variation of wolf diet in Peneda-Gerês study area (Vez/Soajo packs), expressed in Frequency of Occurrence (F.O.) (n – number of prey detections in 118 scats collected during 2008/2010; N - number of scats analysed per season).

	Spring N=28		Summer N=41		Autumn N=22		Winter N=22	
Prey Class	**n**	**F.O. (%)**	**n**	**F.O. (%)**	**n**	**F.O. (%)**	**n**	**F.O. (%)**
Wild Ungulates	**3**	**11%**	**8**	**20%**	**0**	**0%**	**1**	**5%**
C. capreolus	2	7%	6	15%	0	0%	0	0%
S. scrofa	1	4%	2	5%	0	0%	1	5%
Domestic Ungulates	**25**	**89%**	**33**	**80%**	**22**	**100%**	**21**	**95%**
B. taurus	4	14%	7	17%	3	14%	2	9%
E. caballus	18	64%	24	59%	17	77%	18	82%
C. hircus	3	11%	2	5%	2	9%	1	5%
Total	28	100%	41	100%	22	100%	22	100%

Appendix XIII – Overlap of trophic niche (Pianka's Index) of wolf diet in Peneda-Gerês study area.

	Winter	Spring	Summer	Autumn
Winter	-			
Spring	0,983	-		
Summer	0,958	0,986	-	
Autumn	0,995	0,991	0,963	-

Appendix XIV - Number (N Killed) and percentage (%K) of wolf attacks to each livestock species declared to ICNF in the parishes included in Peneda-Gerês study area in comparison with the number of prey detections (N Diet) and respective proportion (% D) in wolf scats, during the time period covered by diet analysis.

	N Killed	% K	N Diet	% D
Sheep	158	11	0	0
Goats	270	18	8	8
Cattle	724	49	16	16
Horses	322	22	77	76
Donkeys	0	0	0	0
Dogs	0	0	0	0
Total	**1474**	**100**	**101**	**100**

Appendix XV – Intra-pack individual variation of wolf diet in four different individuals identified by non-invasive genetics (LAM139; LAM142; LAM143; LAM144) and belonging to the same pack in Peneda-Gerês study area, expressed in Frequency of Occurrence (F.O.) (n – number of prey detections in 14 scats collected in the same day: 6/10/2013; N - number of scats analysed per wolf genetically identified).

	LAM139 N=5		LAM142 N=3		LAM143 N=3		LAM144 N=3	
	n	F.O (%)	n	F.O (%)	n	F.O (%)	n	F.O (%)
B. taurus	0	0	2	50	1	25	0	0
E. caballus	2	40	0	0	2	50	2	67
C. hircus	2	40	1	25	0	0	1	33
C. capreolus	0	0	1	25	0	0	0	0
S. scrofa	0	0	0	0	1	25	0	0
***Felis* sp.**	1	20	0	0	0	0	0	0
Total	**5**	**100**	**4**	**100**	**4**	**100**	**3**	**100**

Appendix XVI – Individual variation of wolf diet along time in one single individual identified by non-invasive genetics (wolf LSD 07) in Sul do Douro study area between 2008 and 2015, expressed in Frequency of Occurrence, F.O. (n – number of prey detections in 15 scats collected between 2008 and 2015; N - number of scats analysed per year).

	2008 N=2		2009 N=1		2011 N=4		2013 N=1		2014 N=6		2015 N=1	
	n	**F.O (%)**	**n**	**F.O (%)**	**n**	**F.O (%)**	**n**	**F.O (%)**	**n**	**F.O (%)**	**n**	**F.O (%)**
S. scrofa	1	33	0	0	0	0	0	0	0	0	1	100
O. aries	0	0	0	0	3	60	0	0	0	0	0	0
C. hircus	0	0	0	0	0	0	0	0	1	17	0	0
Lagomorphs	1	33	0	0	2	40	1	100	4	67	0	0
Galliformes	0	0	0	0	0	0	0	0	1	17	0	0
M. meles	1	33	1	100	0	0	0	0	0	0	0	0
Total	**3**	**100**	**1**	**100**	**5**	**100**	**1**	**100**	**6**	**100**	**1**	**100**

Appendix XVII – Individual variation of wolf diet along time in one single individual identified by non-invasive genetics (wolf LSD 53) in Sul do Douro study area between 2011 and 2015, expressed in Frequency of Occurrence, F.O. (n – number of prey detections in 16 scats collected between 2011 and 2015; N - number of scats analysed per year).

	2011 N=1		2012 N=5		2013 N=2		2014 N=4		2015 N=4	
	n	**F.O (%)**	**n**	**F.O (%)**	**n**	**F.O (%)**	**n**	**F.O (%)**	**n**	**F.O (%)**
S. scrofa	0	0	0	0	0	0	0	0	1	20
E. caballus	0	0	0	0	0	0	1	14	0	0
S. domestica	0	0	0	0	0	0	1	14	0	0
Lagomorphs	1	50	5	80	2	100	4	57	4	80
Galliformes	1	50	1	20	0	0	1	14	0	0
Total	**2**	**100**	**6**	**100**	**2**	**100**	**7**	**100**	**5**	**100**